Agronomic, quality, and genetic analysis of the *Rht18* semi-dwarfing gene in a Canadian Western Red Spring Wheat (*Triticum aestivum* L.) background

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ABSTRACT

Semi-dwarf wheat has historically resulted in reduced stem lodging, increased harvest index, and in some areas, improved yields. The *Rht18* gene is known to reduce height in wheat, but there are currently no commercial varieties in Western Canada that contain this gene. In Western Canada the two main sources of dwarfing in wheat, *Rht-B1b* and *Rht-D1b*, have negative agronomic effects, such as a reduced coleoptile length, reduced emergence, and varietal off-types. Therefore, it is favorable to investigate the introduction of *Rht18* for use in Canadian varieties. This was carried out by measuring the agronomic and quality differences between two set of near-isogenic lines with the same genetic background, but one carrying the gene and the other not. The *Rht18* gene resulted in dwarfing by approximately 16.2 cm compared to the regular height NILs. It showed the expected benefits of reduced stem lodging and increased harvest index. There was an increase in days to flowering and maturity, spikes per unit area, protein percentage, and Falling Number, as well as a minor reduction in yield, test weight, and thousand kernel weight in the *Rht18*-carrying NILs. There was also reduced coleoptile length but no reduction in emergence compared to NILs that did not carry the gene. Compared to check varieties, these agronomic measurements were within a normal range for Canada Western Red Spring wheat varieties.

The location of *Rht18* was identified and markers were validated to ensure the most efficient breeding and introduction of the gene into the breeding program. The SNPs IWB52666, IWB39455, and IWA1813 were linked to *Rht18* on chromosome 6A of wheat. They were also validated and are appropriate for use in a hexaploid wheat breeding program. Having the availability of these SNPs as molecular markers and knowing the effects of *Rht18* introduction into a hexaploid wheat background will allow for increased breeding potential in the future and the possibility of a new dwarfing source in varieties in Western Canada. Semi-dwarf wheat containing *Rht18* can been grown with higher resistance to stem lodging and harvest index without major linkage drag of agronomic and quality traits.

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LIST OF ABBREVIATIONS

CDC	: Crop Development Centre
CWRS	: Canadian Western Red Spring
FHB	: Fusarium head blight
FN	: Falling Number
GA	: Gibberellic acid
HI	: Harvest index
KASP	: Kompetitive Allele Specific polymerase chain reaction
KCRF	: Kernen Crop Research Farm
LSD	: Fisher's Least Significant Difference
mb	: Moisture basis
NIL	: Near-isogenic line
NIR	: Near-infrared reflectance spectroscopy
PC	: Principal components
PCA	: Principal component analysis
QTL	: Quantitative trait locus
Rht	: *Reduced height*
RIL	: Recombinant inbred lines
SAS®	: Statistical Analysis Software®
SDS	: Sodium dodecyl sulfate
SNP	: Single nucleotide polymorphism
SSR	: simple sequence repeat
VRI	: Visual Rating Index

1.0 Introduction

1.1 Background

Bread wheat (*Triticum aestivum* L.) is a common field crop grown in Western Canada belonging to the *Poaceae* family, along with other common grain crops; durum wheat (*Triticum durum* L.), maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), and oat (*Avena sativa* L.). Wheat (*T. spp.)* is a major cultivated crop in Canada, with approximately 9,406,000 ha being sown in 2016 (Statistics Canada 2016). On a world scale, wheat is the fourth most produced crop, ranked after sugar cane, maize, and rice, with 749,460,077 tonnes produced in 2016 (FAOSTAT 2018).

In Canada the wheat produced for human consumption has a variety of end-uses depending on its market class; including pan breads, flat bread, steamed bread, hearth bread, noodles, cookies, cakes, and pastries (Canadian Grain Commission 2016). With the rapid increase in human population, it is important to have an increase in food production. Wheat contributes approximately 20% of the total dietary calories and protein consumed worldwide, so it is specifically important to develop new varieties of wheat to help feed the growing global population (Shiferaw et al. 2013).

With wheat being such an important field crop, for both economical and dietary reasons, it is important to breed wheat varieties that grow the most efficiently in a given environment and that produce higher yields. Lodging, or the permanent displacement of the plant from a vertical position, can cause reductions in breadmaking quality and yield losses in wheat, ranging from 7-80%. This loss of yield in grain yield caused by a reduction in photosynthetic capacity due to undesired plant architecture, as well as partitioning of dry matter to support the plant structure rather than to the developing spike and grain (Piñera-Chavez et al. 2016b). Agronomically, lodging can complicate harvest, increase susceptibility to diseases and pests, and negatively impact crop development by decreasing grains m^{-2} and average grain weight (Piñera-Chavez et al. 2016a). Along with proper farming practices, growing semi-dwarf wheat has been found to increase stem lodging resistance. Semi-dwarfing genes (commonly symbolized by the abbreviation *Rht*) potentially prevent stem lodging by shortening the height of the plant, increasing straw strength, or other changes in plant structure (Ellis et al. 2004). It is important to

conduct research to assess the agronomic, yield, and quality characteristics of new semi-dwarfing genes to determine whether there is a benefit in using them, to the Western Canadian wheat sector.

The semi-dwarf gene *Rht18* was introgressed into a Western Canadian hexaploid wheat background. The first objective of this study was to test for the presence of linkage drag of the gene and analyze the potential effects of it. This was conducted using agronomic and quality tests on near-isogenic lines (NILs) of the same background, that either carried the gene or did not. The second objective of the study was to develop a Kompetitive Allele Specific polymerase chain reaction (KASP) marker for *Rht18* that could distinguish between the NILs and other wheat cultivars that either did or did not carry the gene.

2.0 Literature Review

2.1 Dwarfing Genes in Wheat

2.1.1 History of semi-dwarf wheat

Two wheat cultivars from Japan, Akakomugi and Daruma, were some of the earliest and most successful semi-dwarf cultivars used in wheat breeding with the dwarfing genes originating from these two cultivars present in most of the semi-dwarf wheat in the world (Gale and Youssefian 1985). It is believed that the dwarfing genes originated in Korea where dwarf cultivars were grown as a wheat mixture and were brought to Japan during the Korean-Japan War (Cho et al. 1993). Japanese cultivars carrying the genes were spread to different parts of the world.

Akakomugi is the original source of the *Rht8* gene. In 1911, Nazareno Strampelli of Italy introduced this dwarfing gene into his own breeding program, and it subsequently became a main component of hexaploid wheat breeding in Italy as well as the rest of Central and Eastern Europe. This cultivar also carried the photoperiod insensitive gene *Ppd-D1*, making it valuable in areas where daylength-insensitive crops are desired (Gale and Youssefian 1985). Akakomugi was crossed with the line Selezione 21 (created from crossing Wilhelmina Tare × Rite), which were high yielding and rust-resistant, respectively. This resulted in cultivars that were early maturing, rust-resistant, high yielding, semi-dwarf, and lodging resistant. Cultivars resulting from this cross spread to many countries, especially China and Argentina, and were grown and used in breeding programs throughout the world (Borojevic and Borojevic 2005; Salvi et al. 2013).

Daruma was another important dwarf wheat from Japan, carrying two of the most commonly used *Rht* genes, *Rht-B1b* (previously *Rht1*) and *Rht-D1b* (previously *Rht2*). At the start of the 20th century, Daruma was crossed with American cultivar Fultz by the Japanese scientist Gonjiro Inazuka, and Norin 10 was derived from the cross between Fultz-Daruma and another American cultivar Turkey Red (Dalrymple 1980; Lumpkin 2015). The two commonly used dwarfing genes, *Rht-B1b* and *Rht-D1b*, were introduced into many breeding programs in the late 1940's from Norin 10, originally by Orville Vogel at Washington State University, who then

gave seed to Norman Borlaug at the Mexico at the International Maize and Wheat Improvement Center (Lumpkin 2015). The resulting semi-dwarf cultivars, specifically those bred by Norman Borlaug in the 1950's and 1960's, allowed for increased yields by increasing the ability for the plant to support the increase in grain weight and therefore reducing stem lodging. This time of increased yields is called the 'Green Revolution' (Hedden 2003). Ever since the start of the 20th century, these three semi-dwarfing genes have become very commonly used throughout the world in multiple hexaploid and tetraploid wheat breeding programs.

Since the success of dwarfing genes in these earlier wheat cultivars, there have been multiple new genes generated using mutagenesis (Konzak 1976). There are currently 25 characterized *Reduced height* (*Rht)* genes (*Rht1-Rht25)* in wheat (McIntosh et al. 2013; Chen et al. 2015; Tian et al. 2017; Mo et al. 2018.). The *Rht18* gene was created by a fast-neutron induced mutation of the durum cultivar Anhinga, resulting in the release of Icaro in 1986. *Rht18* has a semi (incomplete) dominant phenotype (Konzak 1988) and is found on chromosome 6A (Haque et al. 2011; Tang 2015; Vikhe et al. 2017; Ford et al. 2018; Grant et al. 2018).

2.1.2 Gibberellic Acid Sensitivity

Gibberellic acid (GA) is one of the four major gibberellins, which are plant hormones that promote seed germination, stem elongation, leaf expansion, trichome development, pollen maturation, and flowering induction. Semi-dwarfing genes each have an individual, specific mutation that causes a change in the pathway of GA, which results in reduced cell elongation and growth, and therefore dwarfing (Davière and Achard 2013). Semi-dwarf wheat can generally be categorized as either GA sensitive or GA insensitive, where the application of the plant hormone (GA) can cause the dwarfing plant to have either increased cell growth and elongation in GA sensitive plants, or have no effect in GA insensitive plants.

The semi-dwarfing genes *Rht-B1b*, *Rht-D1b*, *Rht-B1c* (formerly known as *Rht3*) and *Rht-D1c* (formerly known as *Rht10*) are all classified as GA insensitive, with the majority of the remaining semi-dwarf alleles being classified as GA sensitive (Konzak 1988). The insensitivity of the *Rht-B1b*, *Rht-D1b*, and *Rht-B1c* genes to GA was first reported by Allan et al. (1959), where wheat carrying these genes did not respond to GA application by stem elongation like the other tall wheats that were evaluated. The semi-dwarf plants that carry the GA insensitive genes are found to have a reduction in height due to mutations in a GA signaling pathway, specifically in the DELLA proteins (Peng et al. 1999).

DELLA proteins are transcriptome regulators that negatively regulate plant growth. When GA is not present within the cell, DELLA proteins repress the plant growth responses that typically occur in the presence of GA. In regular height plants where GA is properly biosynthesized or in GA sensitive individuals when GA is applied, the GA binds to the protein's GID1 receptors. This results in the DELLA proteins degrading and normal plant growth continues (Davière and Achard 2013; Colebrook et al. 2014). In GA insensitive individuals, such as *Rht-B1b* and *Rht-D1b*, there is a mutation in a DELLA protein, preventing the degrading of the protein, therefore preventing normal plant growth in the presence of GA. The mutation for both these alleles create nucleotide substitutions that create stop codons in the N-terminal regions of their respective DELLA proteins (Peng et al. 1999).

Another dwarfing process results from mutations that cause disruptions in the GA biosynthesis pathway, which is common for GA sensitive semi-dwarf plants. In order for normal cell division and elongation, and therefore, full stem elongation within the plant, bioactive forms of the plant hormone GA have to be produced (Colebrook et al. 2014). The GA sensitive gene *Rht18* is believed to be a gain of function mutation in the coding region of *GA2oxA9*, causing increased expression of the gene. *GA2oxA9* is believed to encode a GA 2-oxidase which converts the bioactive intermediates GA_{12} and GA_{53} into the inactive forms GA_{110} and GA_{97}, respectively. This results in a decrease in production of bioactive GA_1, and therefore a reduced height (Ford et al. 2018).

2.2 Effects of *Rht* genes in wheat

2.2.1 Agronomic Effects of Rht genes

Previous to the adaptation of semi-dwarf wheat in breeding programs, some breeders believed that only tall plants had the potential to produce high yields. Semi-dwarf germplasm was introduced in North America in the 1940's, specifically the cultivar Norin 10. Norin 10 was found to have problems with male sterility, outcrossing, stem and leaf rust susceptibility, leaf unfolding prior to emergence from the ground, and shriveled, soft grain with low gluten content (Dalrymple 1980; Lumpkin 2015). After years of breeding and selection the first American semi-dwarf cultivar, Gaines, was released in 1962 (Dalrymple 1980).

Since then there have been both positive and negative agronomic effects of semi-dwarf genes reported on developmental and agronomic characteristics of wheat. Wheat plants carrying semi-dwarf genes have shorter statures compared to the regular height wild-types. Some semi-

dwarf plants also have stronger stems which, along with their reduced height, increase resistance to stem lodging. This allows for a more intensive use of inputs on developing crops, such as increased use of fertilizers and irrigation (Gale and Youssefian 1985). Semi-dwarf wheat plants produce less aboveground biomass and an increase in both harvest index (HI) and yield potential, depending on parental background (Richards 1992a). The potential increases in yield and HI are believed to be due to the plant's decreasing demand for assimilating nutrients in the stem because of its reduced growth, and therefore more assimilate being used in the developing ear (Flintham et al. 1997). This shows the potential of height reduction in wheat cultivar development and the importance of testing specific genes within a specific breeding program in Western Canada.

NILs carrying the GA insensitive genes *Rht-B1b*, *Rht-D1b*, and *Rht-B1c* reduce plant height by approximately 16%, 17%, and 50% compared to their tall controls (Flintham et al. 1997). In one study there was no reduction in the duration of stem growth in semi-dwarf wheat plants, but a reduced rate of elongation due to a decrease in total growth (Youssefian et al. 1992). Due to the increased partitioning of assimilates into the developing spike, lines carrying these three genes have been shown to have an increase in fertile florets and an increase in grain number compared to the tall lines (Youssefian et al. 1992; Flintham et al. 1997). Flintham et al. (1997) also found that this increase in grain number was associated with an overall decrease in mean grain weight, and therefore the effect of the semi-dwarfing genes on grain yield per spike varied between the cultivar background of the lines and specific gene combinations used.

The rate and degree of seedling emergence in wheat cultivars carrying either the *Rht-B1b* or *Rht-D1b* semi-dwarfing genes is reduced when they are sown deep to take advantage of available soil moisture. This is due to the reduced length and width of cells, and thus reduced coleoptile length compared to cultivars not carrying either of the two genes (Schillinger et al. 1998). *Rht-B1b*, *Rht-D1b*, *Rht-B1c*, and *Rht-D1c* all have a reduced coleoptile length compared to their tall counterparts (Addisu et al. 2009).

The dwarfing genes *Rht-B1b* and *Rht-D1b* cause problems during cultivar registration and seed certification due to aneuploidy. Plants carrying these genes can be monosomic (2n = 41) for the 4A and 4D chromosomes carrying the *Rht-B1b* and *Rht-D1b* genes. Since the genes produce shorter stature plants, missing one of the chromosomes carrying either of the genes reduces the dosage effect, resulting in tall off-types (Worland and Law 1985).

In GA insensitive semi-dwarf wheat, plant height was positively correlated with coleoptile length, whereas in GA sensitive wheat, the two traits were not found to be correlated (Fick and Qualset 1976; Beharav et al. 1998; Rebetzke et al. 1999; Rebetzke and Richards 2000). This lack of correlation makes it possible to select for wheat with longer coleoptile lengths and a short stature using GA sensitive genes (Rebetzke and Richards 2000). This difference in coleoptile lengths and seedling emergence observed between GA sensitive and insensitive semi-dwarf wheat introduces the possible benefit of using GA sensitive dwarfing genes, such as *Rht18,* as opposed to the commonly used GA insensitive genes.

Plants with other semi-dwarfing genes that are sensitive to GA, such as *Rht8*, *Rht9*, and *Rht12*, have been found to have significantly longer coleoptiles compared to plants carrying *Rht-B1b* or *Rht-D1b* (Rebetzke et al. 2004). Multiple authors have reported varying effects of GA sensitive dwarf genes in wheat on coleoptile length compared to their tall counterparts. In hexaploid wheat *Rht17*, *Rht19*, and *Rht20* reduced coleoptile lengths, whereas *Rht4*, *Rht5*, *Rht7*, *Rht8*, *Rht9*, *Rht12*, and *Rht13* had little or no effect on coleoptile length (Rebetzke et al. 1999; Ellis et al. 2004; Chen et al. 2013; Rebetzke et al. 2012; Wang et al. 2014a).

The agronomic effects of GA sensitive dwarf genes appeared to vary based on the gene and genetic background of the lines tested. Similar to *Rht-B1b* and *Rht-D1b* carriers, semi-dwarf wheat carrying the GA sensitive genes *Rht4*, *Rht8*, *Rht9*, *Rht12*, and *Rht13* had a higher HI, kernel number, and grain yield compared to tall lines (Rebetzke and Richards 2000; Rebetzke et al. 2012). *Rht5* carriers had a reduced grain number and yield, which was believed to be due to later maturity (Rebetzke et al. 2012). The GA sensitive semi-dwarf wheat which carried any of the *Rht5*, *Rht8*, *Rht12*, and *Rht13* genes also had a significantly lower lodging score compared to their tall counterparts (Rebetzke et al. 2012). A different study found conflicting results in that *Rht12* was found to increase floret fertility and spike dry weight, but also decreased grain size with no change in grain yield (Chen et al. 2013).

2.2.2 Effects on disease resistance

Some common field diseases that affect wheat production include stem rust (caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. and E. Henn.), leaf rust (caused by *P. triticina* Eriks.), stripe rust (caused by *P. striiformis* Westend. f. sp. *tritici*), and Fusarium head blight (FHB) (caused by multiple pathogens, including *Fusarium graminearum* Schwabe teleomorph *Giberella zeae* (Schwein. Petch) and *F. avenaceum* (corda ex Fr.) Sacc.)), to name a few

(DePauw et al. 2011). Resistance genes for many of the pathogens have been bred into commonly grown cultivars, which reduces the need for fungicides to be used. Resistance to wheat stem sawfly (*Cephus cinctus* Nort.), orange wheat blossom midge (*Sitodiplosis mosellana* Géhin), and wheat curl mite (*Aceria tosichella* Keifer) have been incorporated into hexaploid wheat as well (McCallum and DePauw 2008).

FHB is a major fungal disease of wheat in Canada and elsewhere and can cause major yield losses and reductions in grain quality, which is especially important in wheat (Haidukowski et al. 2004). FHB fungi can also produce secondary mycotoxins in infected seed which can negatively affect animal and human health if consumed in feed or food (Visconti 2001). There are fungicides available to help reduce the incidence of FHB and resulting mycotoxins, as well as screening and breeding for resistance to the disease (Haidukowski et al. 2004).

Studies have been conducted on the effects of dwarfing genes on FHB infection. Wheat lines carrying *Rht-B1b* or *Rht-D1b* may exhibit a decrease in Type 1 FHB resistance, which is resistance to initial infection. There was no significant effect of *Rht-D1b* but an increase in Type 2 resistance in lines carrying *Rht-B1b*, which is resistance of spread in the spike. Overall, in lines carrying either *Rht-B1b* or *Rht-D1b* there was an increase in susceptibility to FHB (Srinivasachary et al. 2008; Srinivasachary et al. 2009). These two genes may reduce anther extrusion, which results in an increase in Type 1 FHB susceptibility (He et al. 2016). He et al. (2016) proposed that this reduction in anther extrusion was due to the increased expression of the DELLA proteins and decrease in GA production in these mutants, which results in reduced elongation of the filament (Cheng et al. 2004). It is possible that other dwarfing genes that reduce GA production or sensitivity could also result in decreased anther extrusion, and therefore possibly increase FHB susceptibility.

Plant height is negatively correlated with FHB type 1 susceptibility (Yan et al. 2011). Along with physical attributes, it is also possible that dwarfing genes are pleiotropic or linked to quantitative trait loci (QTL) for FHB resistance (Srinvasachary et al. 2009). Since many dwarfing genes in wheat are a result of a mutation in the GA pathway, either biosynthesis or response, there is a possibility that this could affect susceptibility to disease pathogens. Buhrow et al. (2016) reported that when GA was externally applied to wheat spikes along with *Fusarium. graminearum* spores, there was a significant decrease in fungal spread compared to the control. This same study found that when the hormone was applied with the fungicide paclobutrazol,

there was an additive effect of reduced disease severity. The relationship between dwarfing genes and FHB susceptibility varies. One study that measured type II susceptibility in wheat found that there was no difference in susceptibility between *Rht5*, *Rht-D1b*, *Rht4*, *Rht11*, or *Rht13* and their tall counterparts, and that the semi-dwarf genes *Rht4*, *Rht-8+9*, *Rht-B1b*, and *Rht-B1c* had reduced susceptibility (Yan et al. 2011). There have been no studies conducted on the effect of *Rht18* on FHB susceptibility in wheat.

2.2.3 Effects of Rht genes on Wheat Quality

Since one of the main uses of Canadian Western Red Spring (CWRS) wheat is the production of bread, it is important that any dwarfing genes found in CWRS wheat cultivars do not have negative effects on breadmaking quality characteristics. The GA sensitive dwarfing gene *Rht-B1c* has been shown to reduce sprouting damage in wheat grain by reducing the α-amylase content, as measured by the Falling Number (FN) method (Flintham and Gale 1982). This is believed to be due to the aleurone layer in *Rht-B1c* carriers being able to inhibit the production of α-amylase in the presence of GA, which typically enhances the synthesis of the enzyme (Gale and Marshall 1973). *Rht-B1c* has also been shown to decrease the protein content of the grain by 0.8% while not affecting the sodium dodecyl sulfate (SDS) sedimentation volume (Flintham and Gale 1983). Casebow et al. (2016) reported that on average, the GA insensitive genes *Rht-B1b*, *Rht-D1b*, *Rht-B1c*, and *Rht-D1c* were associated with increased FN values (reduced α-amylase content). This increase in FN in wheat carrying either *Rht-B1b* or *Rht-D1b* was also reported by Gooding et al. (1999). The authors also reported an increase in grain yield, but a decrease in grain nitrogen. Since there typically is a negative correlation between yield and grain nitrogen concentration in wheat, this is not unexpected. There are currently no reports on the effects of *Rht18* on bread wheat end-use quality.

2.3 *Rht18* semi-dwarfing gene

2.3.1 History of Rht18

As previously mentioned, there are 25 *Rht* genes characterized, which are named for either the genetic location of the gene (*Rht-B1b*, found on genome B, homoeologous set 1, allele b) or the order it was characterized in (*Rht1* to *Rht25*) (Börner et al. 1996; McIntosh et al. 2013; Chen et al. 2015; Tian et al. 2017; Mo et al. 2018.). The most recently characterized dwarfing gene in wheat is *Rht25*, characterized in 2018 (Mo et al. 2018.).

The *Rht18* gene was created by a fast-neutron induced mutation of the durum cultivar Anhinga, resulting in the release of Icaro (PI503555) in 1986. *Rht18* is classified as GA sensitive (Konzak et al. 1988). Initially, *Rht18* was categorized as semi (incomplete) dominant, but more recent studies classify it as dominant, specifically in a tetraploid wheat background (Konzak 1988; Tang 2015).

The *Rht18* gene originated in durum wheat but has since been introgressed into hexaploid wheat (Yang et al. 2015; Tang 2015). *Rht18* was introgressed into a hexaploid spring wheat background at the Crop Development Centre (CDC) in Saskatoon, Saskatchewan, Canada by Pierre Hucl. The resulting NILs were created by crossing the CWRS wheat cultivar CDC Utmost with Icaro, and subsequently backcrossed with CDC Utmost for five cycles.

2.3.2 Agronomic and quality effects of Rht18

The *Rht18* gene is not currently used in any wheat cultivars released in Western Canada. There is limited research conducted on this gene so it is important that further work, specifically in relation to wheat production in Western Canada, is conducted. *Rht18* is a dwarfing gene, and the heights and internode lengths of wheat carrying the *Rht18* gene were significantly reduced compared to their tall parents (Yang et al. 2015).

Rht18 is GA sensitive, with a 54.7% response for maximum leaf elongation rate to GA in the durum cultivar Icaro (Ellis et al. 2004). The maximum leaf elongation rate of the first leaf in Icaro was significantly reduced compared to its parent cultivar Anhinga, even after GA was applied to both cultivars. The gene is also sensitive to GA application in hexaploid wheat (Yang et al. 2017). Sensitivity to GA would be expected since the *Rht18* mutation corresponds with a mutation in the coding region of *GA2oxA9*, which is believed to encode a GA 2-oxidase that is involved in the GA biosynthesis pathway (Ford et al. 2018).

The coleoptile length of Icaro was significantly reduced, by approximately 18%, compared to its parent Anhinga (Ellis et al. 2004). There was no difference in either the coleoptile length or root characteristics in winter wheat carrying the *Rht18* gene (Yang et al. 2015). In the same study, when wheat carrying another GA sensitive semi-dwarfing gene, *Rht8* along with *Rht18*, the coleoptile length was significantly reduced but the root number, surface area, and volume were all increased. There was no reduction in coleoptile length for either durum or hexaploid wheat *Rht18* carriers in another study by Tang (2015) when compared to tall

lines. In the same study, durum wheat carrying *Rht18* generally had longer coleoptiles than the hexaploid wheat with the same gene.

Since one of the biggest issues when growing currently available semi-dwarf wheat is the potential reduction in emergence compared to regular height plants, it is important to consider the effect of *Rht18* on seedling emergence. When seeded at either shallow (3 cm in trays and 5 cm in field) or deep (12 cm in trays and in field) depths the tall, *Rht18*, *Rht-D1b*, and double dwarf (*Rht-D1b+Rht18*) lines had a similar emergence (Tang 2015). At shallow depths all of the genotypes had approximately 90% emergence, whereas at 12 cm all of the genotypes had approximately 10% emergence. When the lines were sown at a depth of 9 cm in trays, the *Rht18* and tall lines had the same seedling emergence number, which was statistically higher than the *Rht-D1b* and double dwarf lines (Tang 2015). Tang (2015) stated that their results were potentially affected by soil texture in their tray and field experiments. Thus, it is important to measure seedling emergence of *Rht18* carriers under local soil conditions.

The effect of *Rht18* on yield components, such as spikelet number spike^{-1}, grain number spike^{-1}, and 1000-kernel weight varied depending on genetic background of the parent. When measured at either the whole plant level, or the main stem level, the total biomass was reduced and the HI was increased in hexaploid lines with *Rht18* compared to tall lines (Tang 2015). There was no difference between *Rht18* carriers and tall lines for spike lengths, spike number m^{-1}, grain number plant^{-1}, grain number m^{-1}, grain weight, or grain yield m^{-1} in one study by Tang (2015). In three different winter wheat populations there were varying results for the effect of *Rht18.* Two of the populations had a decrease in spikelet number spike^{-1} and grain number spike^{-1}, but an increase in 1000-kernel weight and the third population had inversed results (Yang et al. 2015). There was no statistical difference in the number of fertile tillers plant^{-1} or grain yield plant^{-1} in any of the three populations, and there was a difference in total biomass and HI for only one population, in which the semi-dwarf lines had a reduction in biomass and increase in HI (Yang et al. 2015). Variation in the results between different experiments and the agronomic components were measured on a per plant basis.

2.3.3. Genetic analysis of Rht18

Information regarding the genetics of a specific gene helps plant breeders better understand the effects of the gene on a specific trait or potential pleotropic effects. This knowledge also helps increase the rate of introgression by allowing the use of molecular markers

for a specific trait in breeding programs. A molecular marker is a fragment of a DNA sequence that is located at a specific position, or locus within a genome. To be of value the marker must be variable enough to detect polymorphisms of different alleles in individual plants. The genetic distance between two markers or one marker and the desired gene of interest can be calculated by measuring the frequency of recombination between the two, which is symbolized by the unit centimorgan (cM) (Appels et al. 2011). A smaller genetic distance between the target loci and marker loci is desired, with a perfect marker having a distance of 0 cM. The greater the recombination frequency between the marker and the gene of interest, the more likely that there will be false positives when the marker is used for screening for a specific gene. Appropriate genetic distance can be decided by the individual plant breeder, but preferably it would be less than 5 cM (Collard and Mackill 2008). For the *Rht18* gene to be deployed in breeding programs in Western Canada and throughout the world it is important to understand the genetic control and location of the gene.

Rht18 is a dwarfing gene that is located near the centromere of chromosome 6A in wheat (Haque et al. 2011; Tang 2015; Vikhe et al. 2017; Ford et al. 2018; Grant et al. 2018). The five dwarfing genes *Rht14*, *Rht16*, *Rht18*, *Rht24*, *Rht25* were initially all believed to be located on chromosome 6A in wheat (Haque et al. 2011; Tian et al. 2017; Mo et al. 2018), but more recently Tang (2015) argued that *Rht16* may not be located on this chromosome and is potentially located on chromosome 5B. The *Rht14* and *Rht24* genes are located in the same chromosomal region as *Rht18* (Tang 2015; Würschum et al. 2017). The most recently characterized dwarfing gene, designated as *Rht25*, is at a different location than *Rht18* on chromosome 6A (Mo et al. 2018).

Rht18 was initially classified as semi-dominant (Konzak et al. 1988) but more recent studies have reported varying results. F_1 durum plants heterozygous for *Rht18* had a height similar to the semi-dwarf parent Icaro, not an intermediate height that would be expected with a semi-dominant semi-dwarfing gene (Tang 2015). In another study by Grant et al. (2018) the F_2 plant phenotypes skewed towards the semi-dwarf Icaro phenotype with only a few plants having a tall phenotype similar to the tall parent. The authors suggested that *Rht18* is dominant in nature and that there are possibly other modifier genes that are involved in regulating height in these crosses. In another study by Mo et al. (2018) in an Icaro/Langdon F_2 population *Rht18* appeared to be semi-dominant, with a -75.5% degree of dominance.

Progress has been made towards narrowing down the location of the gene and it has been reported that the gene is a mutation in the coding region of *GA2oxA9* resulting in an increased expression of the gene (Ford et al. 2018). *Rht18* cosegregates with the *GA20xA9* gene and the two genes were mapped within a 1.8 cM interval on chromosome 6A in a study of two durum recombinant inbred line (RIL) populations (Vikhe et al. 2017; Ford et al. 2018). The semi-dwarf cultivar Icaro, which is the original durum mutant carrying *Rht18,* and Halberd-*Rht18,* a hexaploid wheat with *Rht18,* were mutagenized. The overgrowth plants that were regular height at both ploidy levels were found to have varying mutations in the coding region of *GA2oxA9,* which resulted in a loss or reduced amount of the GA 2-oxidase protein, and therefore increased height closer to that the non-dwarfed parents (Ford et al. 2018).

The simple sequence repeat (SSR) markers S470865SSR4 and barc37 and single nucleotide polymorphism (SNP) marker TdGA2ox-A9 were within a 1.8 cM interval of *Rht18* and showed no recombination with the gene in a 256 RIL mapping population (Vikhe et al. 2017). This interval is flanked by barc118 and IWA4371, with the SNP IWA4371 having only one recombinant with *Rht18* in the RIL population. The two SNP markers IAW4371 and TdGA2ox-A9 were present at a high frequency in durum but not bread wheat in 89 durum and bread wheat accessions that were not known to carry *Rht18,* and therefore could potentially be useful in hexaploid, but not durum breeding programs. The SSR marker S470865SSR4 was not present in the hexaploid or durum wheat accessions that were not known to carry *Rht18* and could potentially be used in hexaploid and durum breeding programs (Vikhe et al. 2017). In a different durum RIL mapping population the SSR marker WMS4608 and SNP marker *csRht18-SNP* co-segregated with *Rht18* (Tang 2015). WMS4608 was present in four hexaploid wheat and three durum wheat cultivars that were not known to carry *Rht18* and *csRht18-SNP* was present in three durum cultivars and no hexaploid cultivars that were screened. The majority of the cultivars that these markers were tested on were from Australia, China, and Middle Eastern countries (Tang 2015), and therefore it is not known whether these markers would be present in Western Canadian wheat cultivars.

3.0 Agronomic analysis of the *Rht18* semi-dwarfing gene in a Canadian Western Red Spring (CWRS) wheat (*Triticum aestivum* L.) background

3.1 Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most commonly grown crops worldwide with 749,460,077 tonnes produced in 2016 (FAOSTAT 2018). Wheat contributes approximately 20% of the total dietary calories and protein consumed worldwide, so it is specifically important to develop new cultivars of wheat to help feed the growing population (Shiferaw et al. 2013). Semi-dwarfing wheat has made an important contribution to yield improvements in the past, especially during the early 20th century in Europe and the mid-20th century in Latin America and Southern Asia during the Green Revolution (Gale and Youssefian 1985; Hedden 2003). The contribution of semi-dwarfing to an increase in wheat grain yield is believed to be due to an increased resistance to lodging as a result of a reduction in plant height. This allows for more intensive use of inputs, such as fertilizers and irrigation (Gale and Youssefian 1985). Introducing semi-dwarfing genes, as well as constant improvement of wheat genetics through traditional breeding, has the potential to increase yields without any loss due to plant architecture.

Within Canada, wheat cultivars are assigned into individual market classes, with each class being based on specific quality and functional characteristics of the harvested seed. CWRS is a class of wheat known for its superior milling and baking quality. Each grade of CWRS has a guaranteed level of protein, making it ideal for producing high volume pan breads (Canadian Grain Commission 2017). With such strict quality characteristics used to define the classes and grade of the harvested wheat seed, it is important to test that the introduction of any new genes does not result in any negative effects on the commonly measured quality characteristics of wheat including test weight, thousand kernel weight, protein percentage, and protein quality.

The commonly used semi-dwarfing genes in Canada *Rht-B1b* and *Rht-D1b* have shown many positive benefits, such as increased yield potential. However, there are potential downfalls to growing wheat cultivars carrying these two genes, such as reduced coleoptile length and seedling emergence (Schillinger et al. 1998), tall off-types during pedigree seed production (Worland and Law 1985), and a reduction in overall FHB resistance (Srinivasachary et al. 2009).

Historical worldwide improvements have shown that there are potential benefits of growing semi-dwarf wheat cultivars. It is important to combine potentially valuable semi-dwarfing genes, such as *Rht18,* with good background genetics and agronomic practices and to conduct intense agronomic and quality tests to ensure that these benefits are present.

3.1.1 Research Hypothesis and Objectives

The hypothesis for the first chapter was that the inclusion of the *Rht18* gene from the donor durum cultivar Icaro, backcrossed into the recurrent parent CDC Utmost, would reduce the height of the resulting lines without the linkage of undesirable traits that would cause a reduction in agronomic, yield, or end-use quality characteristics.

The objective was to analyze the potential effects of genetic linkage of traits inherited from Icaro with the *Rht18* gene by conducting agronomic, yield, and quality tests and then comparing these characteristics between the tall NILS, short NILS, and control cultivars.

3.2 Materials and Methods

3.2.1 Plant materials

A total of 24 NILs were used for agronomic and quality testing. These lines were developed at the CDC in Saskatoon, Saskatchewan by Pierre Hucl. They were created by crossing the spring wheat (hexaploid; 2n = 6x = 42; AABBDD) cultivar CDC Utmost with the durum (tetraploid; 2n = 4x = 28; AABB) cultivar Icaro and subsequently backcrossing with CDC Utmost for five cycles (CDC Utmost*6/Icaro). Icaro is a dwarf durum wheat cultivar carrying the *Rht18* gene, which was obtained from fast-neutron induced mutation of the durum cultivar Anhinga (Konzak 1988). Of these 24 lines, 12 lines were classified as tall height (tall NILs) and 12 lines were classified as short height (short NILs), with the 12 short lines carrying *Rht18*. The four cultivars CDC Utmost, Carberry, Glenn, and CDC Go were used as checks (control cultivars). Carberry and CDC Go carry the semi-dwarfing allele *Rht-B1b* and are considered semi-dwarfing wheat cultivars. Neither CDC Utmost nor Glenn carry a semi-dwarfing allele, but carry the regular height alleles, *Rht-B1a* or *Rht-D1a* (Chen et al. 2016). The short lines included RhtNIL 14005, RhtNIL 14015, RhtNIL 14016, RhtNIL 14019, RhtNIL 14020, RhtNIL 14021, RhtNIL 14022, RhtNIL 14023, RhtNIL 14024, RhtNIL 14027, RhtNIL 14028, and RhtNIL 14029. The tall lines included RhtNIL 14003, RhtNIL 14004, RhtNIL 14006, RhtNIL 14007, RhtNIL 14010, RhtNIL 14011, RhtNIL 14012, RhtNIL 14013, RhtNIL 14014, RhtNIL 14017, RhtNIL 14018, and RhtNIL 14032.

3.2.2 Gibberellic acid sensitivity

Gibberellic acid sensitivity was measured in each of the 24 lines under controlled growth conditions. Ten seeds of the 24 NILs and four check cultivars were planted in pots containing propagation soil mix (Sunshine #3, Sun Gro Horticulture Inc. Agawam, MA, USA) which was covered with a 2.5 cm layer of sand. The growth chamber conditions were 21ºC for 18 hour days and 17ºC for the night period. Once the seedlings had emerged they were watered with 50 mL of either of the following two solutions:

- 3.33 g L^{-1} of 20-20-20 fertilizer (Control)
- 3.33 g L^{-1} of 20-20-20 fertilizer + 8ppm gibberellic acid (GA_3) (Sigma-Aldrich, St. Louis, MO, USA)

The appropriate solution was applied to the plants every second day as a soil drench. Once the third ligule on a plant was visible it was harvested by pulling the whole plant from the potting media. The length from the second ligule to the top of the roots were measured using a handheld metric ruler to the nearest millimeter.

3.2.3 Coleoptile and root length measurements

To measure the coleoptile and root lengths four seeds from each line or check cultivar were placed in the middle of a moist germination paper (Whatman® #1, Sigma-Aldrich, St. Louis, MO, USA), approximately 3 cm apart germ end down, with three replications of each line. The germination paper was rolled as a 'cigar roll' with the final diameter being approximately 2 cm. The paper rolls were supported upright and the bases of the rolls were placed in a tray of water to ensure that the paper stayed moist. The rolls were placed in a growth chamber at a constant temperature of 18ºC in complete darkness. After seven days the length of the coleoptiles and roots was recorded. The coleoptile length was measured using a handheld metric ruler to the nearest millimeter, and only measured in seedlings where the plumule began to emerge from coleoptile. The primary root length (longest root present) and total root length (sum of primary root and seminal root lengths) were also measured using a handheld metric ruler to the nearest millimeter.

3.2.4 Field plot design and management

Yield trial field plots were seeded in 2016 and 2017 with each of the 24 NIL and 4 checks grown in a randomized complete block design with four blocks at three locations. In 2016 the plots were sown on 4 May at the Kernen Crop Research Farm (KCRF), 6 May at the Goodale

Farm, and 15 May at Brown site east of the KCRF. In 2017 the plots were sown on 22 May at the KCRF, 12 May at the Goodale Farm, and 20 May at the Brown site. Each plot was sown at a rate of 1400 seeds per plot in a 4.46m^2 area with five rows and a 20 cm row spacing. The fertilizer blend 28-23-0 was applied in furrow at the recommended rate for wheat of 50 kg ha^{-1} for all location years. The herbicide Velocity m3® (Bayer CropScience Inc.) which contains the active ingredients pyrasulfotole, bromoxynil, and thiencarbazone-methyl, was applied at the recommended rate for wheat of 1 L ha^{-1} post-emergence in all location years. The herbicide Refine® (Dupont™) which contains the active ingredients thifensulfuron methyl and tribenuron methyl and the herbicide Axial® BIA (Syngenta®) which contains the active ingredient pinoxaden were applied post-emergence at the Brown site in 2016. The soil classification at both the KCRF and Brown sites is part of the Sutherland Association, dominantly in the orthic dark brown series, with a clay-clay loam texture. Goodale is part of the Bradwell Association, dominantly in the orthic dark brown series, with a very fine sandy loam texture.

3.2.5 In-field plant development measurements

Field measurements consisted of seedling emergence counts per unit area, spikes per unit area, days to headings, days to maturity, and lodging score. Seedling emergence counts were measured once the plants reached the three-leaf stage by counting the number of seedlings within a one-meter length in both the second and third row of each plot. At maturity the spikes per unit area were counted using the same method, counting the number of spikes within a one-meter length in both the second and third row of each plots. Counts per square meter were calculated using a 20 cm row spacing. The days to heading was recorded once 50% of the plants in a plot had fully headed and the days to maturity was recorded when 90% of the plants in a plot had reached physiological maturity, which was determined when the top 2.5 cm of the peduncle had changed color to yellow. Lodging score was rated using the Belgian lodging rating system at maturity (Equation 3.1), with the lodging index ranging from 0.2 for no lodging to 9.0 for complete lodging of the plot.

Equation 3.1. Belgian lodging scale index. The area of surface lodged (S) was rated from 1 to 9 and intensity of lodging (I) was rated from 1 to 5. This index ranged from 0.2 for no lodging to 9.0 for complete lodging.

Lodging Index = S x I x 0.2

In separate trials the FHB response of the test entries was assessed at the University of Manitoba in Carman, Manitoba with one repetition in 2015, one repetition in 2016, and two repetitions in 2017. This was conducted using the visual rating index (VRI) method outlined in Stack and McMullen (1995), which takes into account the incidence and severity of disease on wheat spikes. Along with the 24 NILs and four check cultivars, the six cultivars AC Morse, AC Vista, CDC Teal, AC Cora, FHB 37, and 5602 HR were used as checks for all years and the cultivar AAC Tenacious was used in 2017.

3.2.6 Plant height and internode lengths

Plant height and specific internode lengths were measured at maturity to assess the effect of the *Rht18* allele on stem elongation. Height was calculated by averaging the heights of the two measurements per plot measured to the nearest centimeter (30 cm from the plot edge, at either end). Internode lengths were measured on approximately 5 plants per plot from a selection of 20 plants per plot pulled prior to harvest. The pulled plants were dried for 24 hours at 36°C using forced air. The internodes were measured individually to the nearest millimeter using a metric ruler with I1L being the length of the top, or first internode directly below the spike, I2L being the penultimate, or second internode length, I3L being the third internode length, I4L being the fourth internode length, and I5L being the bottom, or fifth internode length from the top. The spike length was also measured using a handheld metric ruler to the nearest millimeter.

3.2.7 Agronomic traits

The 20 plants per plot pulled prior to harvest were also used to measure biomass characteristics after they were dried. The average number of seeds per spike, seed yield per spike, and number of spikelets per spike were all counted. The weight of total dried aboveground biomass of the plants and the weight of total seed from the plants were measured and used to calculate the HI (Equation 3.2).

Equation 3.2. Harvest index calculation.

Harvest index = weight of seed (g)/dry weight of total aboveground biomass (g)

The plots were combine harvested and the seed was used to measure the 1000-kernel weight, test weight, and yield. The harvested seed was dried in the same method as the 20 pulled plants, as previously described. The 1000-kernel weight was measured by counting 250 seeds

using a seed counter, weighing the seeds, and then multiplying by four to calculate the weight of 1000 seeds. The test weight was measured as the weight in grams of seed per half-liter volume which was measured using a chondrometer and weighed on a tared digital scale. The yield was measured by weighing total seed harvested per plot.

3.2.8 Seed quality measurements

Protein concentration was measured using near-infrared reflectance spectroscopy (NIR) on whole grain seed. Protein concentration measurement by combustion nitrogen analysis, alpha-amylase activity measurement by the FN test, and protein quality measurement by the SDS sedimentation test were conducted on ground seed samples at a 14% moisture basis (mb). Seed samples from each replication were separately ground through a 1.0 mL sieve using a UDY Corporation Cyclone sample mill (Fort Collins, Colorado, USA).

Moisture content of the ground samples was measured using a modified version of the American Association of Cereal Chemists International (AACCI) approved method 44-15.02 (AACC International 1999a). This was conducted by weighing small, heavy gauge aluminum moisture dishes, taring, and then weighing 3 g of ground wheat seed samples into the dishes. Uncovered dishes were placed in an Isotemp 100L Oven FA (Fisher Scientific, Langenselbold, Germany) at 130 degrees Celsius for 65 minutes. Dishes were removed and placed at room temperature with lids on for 20 minutes until cooled. Dishes with ground samples were weighed and moisture content was calculated using Equation 3.3. For the seed quality measurements, the required weights were expressed at a 14% mb, and the weight of ground sample to measure per gram at the desired 14% mb was calculated (Equation 3.4). Averages were calculated by measuring the moisture content of 15 random samples. The moisture content was measured for each of the three sites individually.

Equation 3.3. Moisture percentage of ground wheat samples.
Moisture Percentage = 100 - (weight of dried sample/weight of pre-dried sample) x 100%

Equation 3.4. Calculating the per gram basis on ground wheat sample on a 14% moisture basis (mb). The actual moisture percentage is the moisture percent that was calculated in Equation 3.3.
Weight measured per gram at 14% mb (g) = (100 – 14)/(100-actual moisture percentage)

3.2.8.1 Grain protein concentration analysis

The protein concentration of intact whole grain seed of the 24 NILs and four checks at each location was measured. Near-infrared reflectance spectroscopy analysis was conducted using a NIRSystems 6500 (FOSS, Hillerød, Denmark) instrument. The reflectance spectra were

scanned and recorded from 400 to 2500 nm using WinISI II Project Manager v1.50 Software (FOSS, Hillerød, Denmark). Approximately 25 mL of whole grain was placed in the cell, covered, and placed in the instrument for scanning.

The protein concentrations from the NIR measurements were calibrated using measurements from combustion nitrogen analysis which was conducted using LECO® model FP-528 nitrogen/protein analyzer (LECO Corporation, Saint Joseph, MI, USA). Analysis was performed on 250 mg of ground wheat sample using the AACCI approved method 46-30.01 (AACC International 1999b).

3.2.8.2 Alpha-amylase activity

Alpha-amylase activity, which is used as a measurement of the degree of sprouting or pre-harvest germination in grain, was measured using the FN test on ground seed samples. A modified version of the AACCI approved method 56-81.03 (AACC International 1999d) was followed using a FN apparatus made by Perten Instruments AB (Hägersten, Sweden). This was conducted by measuring 7.0 g (14% mb) of ground sample and putting it into a viscometer tube, followed by 25 mL ddH_2O. A rubber stopper was placed on the tube and the sample was mixed into a homogeneous suspension for five seconds using a Shakematic automatic sample mixer (Perten Instruments AB, Hägersten, Sweden), modified from the AACCI method that states to shake tube 20-30 times upright by hand. Viscometer-stirrers were used to scrape slurry down from the upper part of the tube and inserted into the tubes and then the tubes were placed inside the FN cassette apparatus and the lid was closed. The amount of time in seconds for the plunger to fully move through the sample was recorded as the FN.

3.2.8.3 Protein quality

Protein quality was determined by the ability of gluten proteins in the sample to swell in the presence of lactic acid and was measured using the SDS Sedimentation test. The method conducted is similar to the AACCI approved method 56-61.02 (AACC International 1999c). This was conducted by adding 5.0 g (14% mb) of ground wheat sample and 50 mL of distilled water into a 100 mL test tube and shaken vigorously until all the meal came off the bottom of the tube. Then a glass cap lid was placed in the tube and placed on a mechanical shaker. After 4 minutes, 50 mL of SDS solution (2% lactic acid and 2% SDS in water) was added and the tube was shaken for another 6 minutes using the mechanical shaker. After 6 minutes the tubes were set on a flat surface. The volume of solution in the tube after 20 minutes was recorded.

3.2.9 Data analysis

Statistical analysis was performed using Statistical Analysis Software® (SAS®) version 9.4 (SAS Institute, Inc. Cary, NC, USA). The residual variances for all classes were tested for normality using the PROC UNIVARIATE procedure and the Normal option, with normally distributed residual variance showing a $P > 0.05$ for the Shapiro-Wilk test. They were also tested for homogeneity using the PROC GLM procedure by using the HOVTEST option in the MEANS statement, with homogenous residual variance having shown a $P > 0.05$ for the Levene's test.

For the GA sensitivity test the TTEST procedure was used to compare two group means with the CLASS statement containing the treatment groups (0 ppm or 8 ppm GA_3) and the VAR statement containing the individual seedling measurements. This analysis was performed individually for each line/cultivar with an individual pot as the experimental unit.

For the coleoptile length, root length, in-field development, plant height, internode lengths, agronomic, and seed quality data the PROC MIXED procedure with the Fisher's Least Significant Difference (LSD) test option was used to measure least square means of the fixed effects. Means were considered statistically significantly different at $P < 0.05$. The fixed effects included line and entry nested within line, with all other effects considered random. Heterogeneous variances were modelled in the PROC MIXED procedure with the repeated/group=effect statement (Littell et al. 2006). The mean separation output was converted into letter groupings using the macro program Pdmix800.SAS (Saxton 1998).

NIR measurements were calibrated using LECO® protein percentages analysis for one block for each of the three locations. The correlations between the NIR and LECO® results were highly significant with a Pearson's correlation coefficients of 0.90 ($P < 0.0001$) using the PROC CORR function. The PROC TTEST procedure with the PAIRED statement was used to confirm the calibration of NIR method with the LECO® method of protein analysis (no difference between procedures, $P > 0.05$).

Correlation coefficient and principal component analysis (PCA) was conducted between the 18 variables measured, including yield, test weight, thousand-kernel weight, days to heading, days to maturity, seedling emergence count m^{-2}, spike count m^{-2}, lodging score, spike length, FHB infection, height, HI, spikelet number $spike^{-1}$, seed number $spike^{-1}$, seed yield $spike^{-1}$, grain protein content, FN, and SDS Sedimentation volume. The correlation coefficient and the PCA

were calculated using the means of the 24 NILs. The correlation between the variables was conducted using the PROC CORR function using a Pearson's correlation coefficient calculated at a $P < 0.05$ significance level. The PCA analysis was conducted using the PROC PRINCOMP function.

3.3 Results

3.3.1 Gibberellic acid sensitivity

Carberry, the tall NILs, and the shorts NILs all differed significantly when compared to the control plants that received no GA_3 application and the treated plants that received 8 ppm of GA_3 (Table 3.1). The three checks CDC Utmost, Glenn, and CDC Go did not show a statistical difference between the control and treatment plants. The short NILs carrying *Rht18* had the largest response to GA_3 application with an 84.5% change in seedling length between the plants receiving and not receiving the GA_3 treatment.

Table 3.1. Gibberellic acid sensitivity measurements of CDC Utmost, Carberry, Glenn, CDC Go, tall NILs (not carrying *Rht18*) and short NILs (carrying *Rht18*). Sensitivity to GA_3 was measured between plants receiving treatments of either 0 ppm (check) or 8 ppm of GA_3. Plant height was measured as length from the second ligule to the top of the roots once the plant third ligule was visible on the plant.

		Plant Height (mm)		
Cultivar	**n**	**0 ppm GA_3**	**8 ppm GA_3**	**Percent Change (%)**
CDC Utmost	4	75.2 ± 4.50	97.4 ± 2.60	29.5^{ns}
Carberry	4	55.5 ± 0.30	73.8 ± 0.75	33.0**
Glenn	4	61.1 ± 4.90	89.1 ± 4.35	45.8^{ns}
CDC Go	4	66.6 ± 4.95	77.7 ± 0.85	16.7^{ns}
CDC Utmost*6/Icaro (-Rht18)	48	64.9 ± 1.11	98.2 ± 1.47	51.3****
CDC Utmost*6/Icaro (+Rht18)	48	53.4 ± 0.62	98.5 ± 1.35	84.5****

Note: Data are the mean estimates ± SEM (standard error means) of each genotype. The percent change between the control and 8 ppm treatment within each cultivar is recorded. *, **, ***, ****: significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$, respectively. ns: not statistically significant.

3.3.2 Coleoptile and root length measurements

The short NILs had an average coleoptile length of 58.6 mm, which was statistically shorter than the tall NILs with a length of 64.1 mm. (Table 3.2). The short NILs and tall NILs had similar primary root lengths and total root length. There was no significant difference among the 12 NIL entries that make up either the tall NILs or the short NILs for coleoptile, primary root length, or total root length (Appendix A). CDC Utmost and Glenn did not differ for coleoptile length but were longer ($P < 0.001$) than Carberry and CDC Go. Eleven of the 12 tall NILs had statistically the same coleoptile length as CDC Utmost and Glenn, whereas 11 and 10 of the short NILs had statistically the same coleoptile length as CDC Go and Carberry, respectively.

Carberry, Glenn, and CDC Go had a statistically longer primary root length than all of the tall NILs, short NILs, and CDC Utmost. CDC Go, Carberry, and Glenn had the longest total root length as well, with each having had a varied level of significant difference compared to individual NILs (Appendix A).

Table 3.2. Average coleoptile length (mm), primary root length (mm), and total root length (mm). The tall (*Rht18* not present) NILs of Utmost*6/Icaro and the short (*Rht18* present) NILs of Utmost*6/Icaro were analyzed together. The recurrent parent CDC Utmost, and the three checks Carberry, Glenn, and CDC Go were analyzed together with the individual 24 NILs (complete data in Appendix A).

Lines	n	Coleoptile length (mm)	Primary root length (mm)	Total root length (mm)
Utmost*6/Icaro (- *Rht18*)	36	64.1a	118.3a	281.6a
Utmost*6/Icaro (+ *Rht18*)	36	58.6b	119.5a	283.5a
CDC Utmost	3	67.3	121.2	287.3
Carberry	3	56.8	136.3	361.7
Glenn	3	68.5	149.3	338.7
CDC Go	3	57.5	145.6	389.7
SEM for NILs		0.68	4.01	14.64
CV for NILs		8.61	9.99	15.70
Between NIL groups		****	ns	ns
Within NIL groups		ns	ns	ns
SEM for Checks and NILs		2.41	7.61	27.10
CV for Checks and NILs		8.95	12.07	17.54
For Checks and NILs		****	**	*

Note: Data are the mean estimates of each genotype. Means followed by the same letter within columns are not statistically significantly different based of Fisher's least significant differences (LSD) at $P < 0.05$. SEM is the standard error mean. *, **, ***, ****: significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$, respectively. ns: not statistically significant.

3.3.3 In-field plant development measurements

The combined 2016 and 2017 agronomic measurements of days to heading, days to maturity, spike count per unit area, and lodging score were all characterized by significant differences between the tall and short NILs (Table 3.3). There was no difference in seedling emergence between tall and short NILs as measured at the 3-leaf stage. There was also no difference in emergence between the NILs and checks (Appendix B). The short NILs headed and matured approximately two days later than the tall NILs. Carberry, Glenn, and CDC Go all headed earlier than both NIL groups and CDC Utmost, but Carberry and Glenn matured later than the tall NILs, CDC Utmost, and most of the short NILs. At maturity, the short NILs had a 3.0% increase relative to the tall NILs in spikes per unit area. The short NILs did not lodge at

Table 3.3. Average in field agronomic measurements including days to heading, days to maturity, emergence count, spike count, and lodging score. The tall (*Rht18* not present) NILs of Utmost*6/Icaro, and the short (*Rht18* present) NILs of Utmost*6/Icaro were analyzed together. The recurrent parent CDC Utmost, and the three checks Carberry, Glenn, and CDC Go were analyzed together with the individual 24 NILs (complete data presented in Appendix B). The data is for the KCRF, Brown, and Goodale locations combined over two years (2016 and 2017).

Lines	n	Heading (days)	Maturity (days)	Emergence (plants m^{-2})	Spike Count (spike m^{-2})	Lodging (Belgian scale)
Utmost*6/Icaro (-*Rht18*)	288	53.1b	91.2b	245.6a	553.2b	0.96a
Utmost*6/Icaro (+*Rht18*)	288	55.5a	93.5a	243.0a	569.0a	0.20b
CDC Utmost	24	53.2	92.2	239.2	579.2	1.67
Carberry	24	49.9	95.1	239.2	572.8	0.22
Glenn	24	50.4	94.8	244.1	585.5	0.59
CDC Go	24	49.0	90.8	235.5	589.8	0.27
SEM for NILs		1.21	1.00	8.18	18.84	0.146
CV for NILs		5.23	3.29	17.32	13.10	164.62
Between NIL groups		****	****	ns	**	****
Within NIL groups		****	****	ns	*	ns
SEM for Checks and NILs		1.23	1.07	10.32	21.17	0.267
CV for Checks and NILs		5.91	3.44	17.00	13.33	167.83
For Checks and NILs		****	****	ns	**	****

Note: Data are the mean estimates of each genotype. Means followed by the same letter within columns are not statistically significantly different based on Fisher's least significant differences (LSD) at $P < 0.05$. SEM is the standard error mean. *, **, ***, ****: significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$, respectively. Ns: not statistically significant. Lodging was only present at Goodale and KCRF in 2016 and Goodale in 2017, and varied in severity between blocks.

any of the locations in both years, which was an improvement over the tall NILs. Carberry and CDC Go had similar lodging scores to the short NILs, while CDC Utmost was similar to the tall NILs. The nature of lodging data and the attendant high CV limited meaningful statistical comparisons. Lodging was only reported at the KCRF and Goodale sites in 2016 and Goodale site in 2017 as there was limited expression at the other sites.

There were some differences among the 12 entries within each of the NIL groups for days to heading, days to maturity, and spike number per unit area, which indicated that there were some entries within a given NIL group that deviated from other NILs (Appendix B). The tall NILs had statistically similar measurements for days to heading, days to maturity, emergence count, spike count, and lodging compared to CDC Utmost. The short NILs had, statistically, the same emergence counts and spike counts as CDC Utmost.

The tall NILs had significantly lower levels of FHB infection ($P < 0.001$) than the short NILs, with a VRI of 42.6 for the tall NILs compared to 67.0 for the short NILs (Table 3.4). There was no significant difference in FHB infection among the 12 entries within each NIL group. The tall NILs were similar to CDC Utmost, whereas the short NILs were similar to CDC Go and CDC Teal for FHB infection (Appendix C). The high CV limited meaningful statistical comparisons.

3.3.4 Plant height and internode lengths

The short NILs, at 75.5 cm, were approximately 16.2 cm shorter in height than the tall NILs, which were on average 91.7 cm tall (Table 3.5). This reduction in height was a result of a reduction in spike length, as well as all of the 5 internodes (Figure 3.1). The top internode (internode 1) had the greatest percent reduction in length between the tall and short NILs, with each lower internode having a sequentially smaller percent reduction. The top internode was 88.8 mm shorter for the short NILs compared to the tall NILs (Table 3.5), which was responsible for over half of the total height reduction.

There was a significant difference within the NIL groupings for the plant height, spike length, and the top four internodes as well (Appendix D). Even though there were differences within the NIL groups, the plant height and top four internodes had no overlapping lengths between individual tall NILs and short NILs. For the spike length there was an overlap between the individual NILs in the groups, where some of the short NILs had longer spike lengths than some of the tall NILs (Appendix D). The short NILs were shorter in height than all of the check

Table 3.4. Average Fusarium head blight (FHB) percentage on wheat spikes based on the visual rating index (VRI). The tall (R*ht18* not present) NILs of Utmost*6/Icaro and the short (*Rht18* present) NILs of Utmost*6/Icaro were analyzed together. The recurrent parent CDC Utmost, and the 10 checks Carberry, Glenn, CDC Go, AC Morse, AC Vista, CDC Teal, AC Cora, FHB 37, 5602 HR, and AAC Tenacious were analyzed together with the individual 24 NILs (complete data presented in Appendix C). The data is for the University of Manitoba inoculated nursery in Carman, Manitoba combined over three years (2015, 2016, and 2017).

Lines	n	FHB (%)
Utmost*6/Icaro (- *Rht18*)	48	42.6b
Utmost*6/Icaro (+ *Rht18*)	48	67.0a
CDC Utmost	4	46.7
Carberry	4	15.2
Glenn	4	13.2
CDC Go	4	67.9 ± 18.78
AC Morse	4	55.0
AC Vista	4	52.5
CDC Teal	4	61.7
AC Cora	4	17.5
FHB 37	4	13.7
5602 HR	4	20.2
AAC Tenacious	2	6.0 ± 14.68
SEM for NILs		9.95
CV for NILs		40.88
Between NIL groups		***
Within NIL groups		ns
SEM for Checks and NILs		12.42
CV for Checks and NILs		314.78
For Checks and NILs		****

Note: Data are the mean estimates of each genotype. Means followed by the same letter within columns are not statistically significantly different based on Fisher's least significant differences (LSD) at $P < 0.05$. SEM is the standard error mean. AAC Tenacious and CDC Go had a different SEM when analyzed with all the individual lines. This is due to reduced sample size of AAC Tenacious and the high variation in percent FHB at different site years for CDC Go. *, **, ***, ****: significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$, respectively. ns: not statistically significant.

Table 3.5. Average measurements of plant height, spike length, top internode length (I1L), penultimate or second internode length (I2L), third internode length (I3L), fourth internode length (I4L), and bottom or fifth internode length (I5L). The tall (*Rht18* not present) NILs of Utmost*6/Icaro, and the short (*Rht18* present) NILs of Utmost*6/Icaro were analyzed together. The recurrent parent CDC Utmost, and the three checks Carberry, Glenn, and CDC Go were analyzed together with the individual 24 NILs (complete data presented in Appendix D). The data is for the KCRF, Brown, and Goodale locations combined over two years (2016 and 2017).

Line	n	Plant Height (cm)	Spike Length (mm)	I1L (mm)	I2L (mm)	I3L (mm)	I4L (mm)	I5L (mm)
Utmost*6/Icaro (- *Rht18*)	288	91.7a	77.3a	365.1a	227.9a	154.0a	100.2a	29.3a
Utmost*6/Icaro (+ *Rht18*)	288	75.5b	76.6b	276.3b	183.1b	132.3b	85.6b	27.7b
CDC Utmost	24	92.8	78.0	373.6	232.0	155.8	101.0	29.6
Carberry	24	83.2	75.1	364.3	195.5	126.0	86.1	28.5
Glenn	24	92.3	79.1	387.1	218.4	147.0	97.1	27.7
CDC Go	24	83.1	74.5	358.0	193.4	121.5	81.4	29.7
SEM for NILs		3.57	4.22	7.57	8.19	13.30	13.92	1.62
CV for NILs		12.76	6.60	15.94	15.63	16.29	20.90	22.39
Between NIL groups		****	****	****	****	****	****	***
Within NIL groups		****	****	**	***	***	***	ns
SEM for Checks and NILs		3.48	4.17	8.48	8.32	12.67	13.53	2.37
CV for Checks and NILs		5.91	6.66	15.73	15.14	16.35	20.66	23.09
For Checks and NILs		****	****	****	****	****	****	*

Note: Data are the mean estimates of each genotype. Means followed by the same letter within columns are not statistically significantly different based on Fisher's least significant differences (LSD) at $P < 0.05$. SEM is the standard error mean. *, **, ***, ****: significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$.

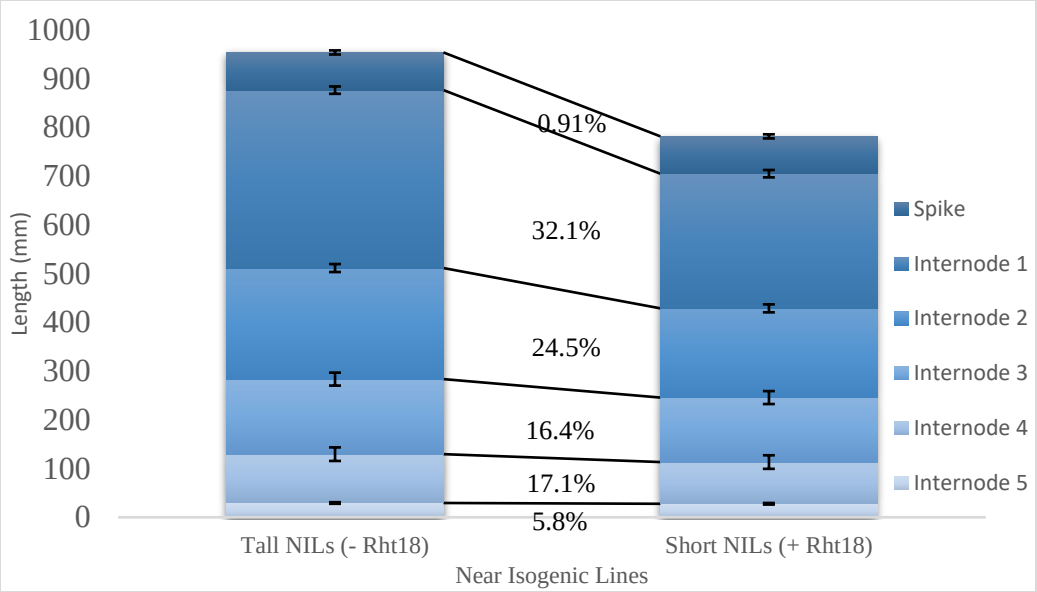

Figure 3.1. Comparing the length of the spikes and five internodes of the tall NILs (Utmost*6/Icaro) that do not carry the *Rht18* allele to the short NILs (Utmost*6/Icaro) that do carry the *Rht18* allele. The percentages show the reduction of the spike and each individual internode of the short NILs compared to the tall NILs.

cultivars including Carberry and CDC Go, which both carry the semi-dwarfing allele *Rht-B1b*, and were 83.2 and 83.1 cm in height, respectively.

3.3.5 Agronomic traits

There were significant differences between the short NILs and tall NILs for all of the agronomic traits evaluated (Table 3.6). The short NILs had a reduction in yield, test weight, thousand kernel weight, number of spikelets spike^{-1}, number of seeds spike^{-1}, and seed yield spike^{-1}, but an increase in HI compared to the tall NILs. Although statistically significant, the differences in test weight, thousand kernel weight, spikelets spike^{-1}, seeds spike^{-1}, and seed yield spike^{-1} were relatively small.

The short NILs yielded 4318 kg ha^{-1}, approximately 100 kg ha^{-1} less than the tall NILs. It is important to note that there were significant differences within the NIL groups as well as overlap between the two NIL groupings, with a yield range of 4270 to 4600 kg ha^{-1} for the tall NILs and 4233 to 4435 kg ha^{-1} for the short NILs (Appendix E). The short NILs had a test weight of 78.0 kg hl^{-1}, which was 1.3 kg hL^{-1} less than the tall NILs (Table 3.6). The short NILs had a 2.9% reduction in thousand kernel weight compared to the tall NILs. Even though there were significant differences between the NILs within each NIL grouping for both the test weights and thousand kernel weights, each NIL group clustered together, with the tall NILs

exhibiting an overall increase in both traits, compared to the short NILs (Appendix E).

The short NILs had fewer spikelets spike^{-1} ($P < 0.001$) than the tall NILs (Table 3.6). There was also a statistically significant 8.5% reduction in the number of seeds spike^{-1} and 11.1% reduction in seed yield spike^{-1} in the short NILs compared to the tall NILs. The short NILs had a HI of 0.384 which was statistically higher ($P < 0.001$) than that of the tall NILs, which had a mean HI of 0.376 (Table 3.6). Similar to the test weights and thousand kernels weight, there was a significant difference between NILs within each NIL grouping, and each of the groups clustered together with the individual tall NILs having an overall increased number of spikelets spike^{-1}, number of seeds spike^{-1}, and seed yield spike^{-1} and an overall decrease in HI compared to the individual short NILs (Appendix E).

Even though the short NILs showed a reduction in the majority of agronomic traits compared to the tall NILs, they are within the range compared to other wheat cultivars (Appendix E). All, or a majority of, the individual short NILs had similar yields relative to CDC Utmost, Carberry, and Glenn; the same TKW as Glenn; more spikelets spike^{-1} than Carberry, Glenn, and CDC Go; the same seed spike^{-1} as Carberry, Glenn, and CDC Go; the same seed yield spike^{-1} as Glenn; and the same HI as Carberry, CDC Go, and CDC Utmost but higher HI than Glenn.

3.3.6 Seed quality measurements

There was a significant difference in the protein percentage, FN, and SDS sedimentation volume between the short and tall NILs (Table 3.7). The short NILs had a higher grain protein content and FN compared to the tall NILs. The tall NILs had a higher SDS sedimentation volume compared to the short NILs. It is important to note that even though the short NIL grouping had an overall, statistically significant, higher protein percentage than the tall NILs, there was a significant difference between NILs within the same group as well, and protein content showed no clustering trend of measurements into the two groups (Appendix F). The short NILs had a higher ($P<0.0001$) FN than the checks, and the majority of the short NILs also had a higher SDS Sedimentation volume than Carberry and CDC Go.

3.3.7 Association of Agronomic and Quality Variables between NILs

Using the means for the 12 tall and 12 short NILs, height had a positive correlation with yield, test weight, thousand kernel weight, lodging score, spike length, spikelet number spike^{-1}, seed number spike^{-1}, seed yield spike^{-1}, and SDS sedimentation volume and a negative

Table 3.6. Average post-harvest agronomic measurements including yield, test weight (TW), thousand kernel weight (TKW), number of spikelets spike^{-1}, number of seeds spike^{-1}, seed yield spike^{-1}, and harvest index (HI). The tall (*Rht18* not present) NILs of Utmost*6/Icaro and the short (*Rht18* present) NILs of Utmost*6/Icaro were analyzed together. The recurrent parent CDC Utmost, and the three checks Carberry, Glenn, and CDC Go were analyzed together with the individual 24 NILs (complete data shown in Appendix E). The data is for the KCRF, Brown, and Goodale locations combined over two years (2016 and 2017).

Line	n	Yield (kg ha^{-1})	TW (kg hl^{-1})	TKW (g)	Spikelets Spike^{-1}	Seeds Spike^{-1}	Seed Yield Spike (g) $^{-1}$	HI
Utmost*6/Icaro (- *Rht18*)	288	4423a	79.3a	34.1a	15.1a	25.9a	0.860a	0.376b
Utmost*6/Icaro (+ *Rht18*)	288	4318b	78.0b	33.1b	14.8b	23.7b	0.765b	0.384a
CDC Utmost	24	4475	79.4	34.4	15.2	26.3	0.896	0.383
Carberry	24	4542	80.9	33.9	14.0	23.8	0.795	0.397
Glenn	24	4472	83.0	32.9	14.2	24.4	0.788	0.353
CDC Go	24	4632	80.0	37.9	13.3	23.4	0.863	0.412
SEM for NILs		321.8	2.87	0.88	0.29	2.26	0.0885	0.0139
CV for NILs		13.52	3.83	5.03	5.14	17.07	16.56	11.29
Between NIL groups		****	****	****	****	****	****	***
Within NIL groups		***	****	****	****	*	*	**
SEM for Checks and NILs		320.4	2.83	0.84	0.30	2.32	0.0896	0.0147
CV for Checks and NILs		13.75	3.93	5.47	5.80	16.98	16.78	11.42
For Checks and NILs		****	****	****	****	****	****	****

Note: Data are the mean estimates of each genotype. Means followed by the same letter within columns are not statistically significantly different based on Fisher's least significant differences (LSD) at $P < 0.05$. SEM is the standard error mean. *, **, ***, ****: significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$.

Table 3.7. Average seed quality measurements including percent protein, Falling Number (FN), and SDS Sedimentation (SED). The tall (*Rht18* not present) NILs of Utmost*6/Icaro and the short (*Rht18* present) NILs of Utmost*6/Icaro were analyzed together. The recurrent parent CDC Utmost, and the three checks Carberry, Glenn, and CDC Go were analyzed together with the individual 24 NILs (complete data shown in Appendix F). The data is for the KCRF, Brown, and Goodale locations combined over two years (2016 and 2017).

Line	n	Protein (%)	FN (seconds)	SED (ml)
Utmost*6/Icaro (- *Rht18*)	288	16.34b	440.5b	81.3a
Utmost*6/Icaro (+ *Rht18*)	288	16.46a	456.4a	78.3b
CDC Utmost	24	16.38	437.7	80.3
Carberry	24	16.29	387.8	76.8
Glenn	24	16.43	414.0	80.7
CDC Go	24	16.51	418.5	76.5
SEM for NILs		0.127	5.77	0.77
CV for NILs		2.70	5.70	4.97
Between NIL groups		****	****	****
Within NIL groups		****	****	****
SEM for Checks and NILs		0.136	6.58	1.30
CV for Checks and NILs		2.81	6.40	5.41
For Checks and NILs		****	****	****

Note: Data are the mean estimates of each genotype. Means followed by the same letter within columns are not statistically significantly different based on Fisher's least significant differences (LSD) at $P < 0.05$. SEM is the standard error mean. *, **, ***, ****: significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$

correlation with days to heading, days to maturity, spike count m^{-2}, FHB rating, HI, and FN (Table 3.8).

All of the seed yield traits, including grain yield (kg ha^{-1}), test weight, thousand kernel weight, spikelet number $spike^{-1}$, seed number $spike^{-1}$, and seed yield $spike^{-1}$ were positively correlated with each other. The amount of lodging was positively correlated with all of these seed yield variables except for grain yield (kg ha^{-1}). Harvest index did not have a significant correlation with grain yield (kg ha^{-1}) or spike specific seed yield characteristics, but did have a significant negative correlation of coefficient of -0.50 with test weight and thousand kernel weight.

For the quality analysis, grain protein content had a significant negative correlation with thousand kernel weight and a positive correlation with FN. Even though FN and SDS sedimentation values were not correlated to each other, their correlation with other variables were inverse of each other for 15 of the 16 variables.

Table 3.8. Pearson **c**orrelation coefficient between 18 agronomic, anatomical, and quality variables for 24 hexaploid wheat near-isogenic lines. Of the 24 NILs, 12 are short NILs carrying *Rht18* and 12 are tall NILs not carrying *Rht18*. The data is for KCRF, Brown, and Goodale locations combined in 2016 and 2017.

Variable	Y	TW	TKW	DTH	DTM	Seedl	SPK	LOD	SPKL	FHB	HT	HI	SKSP	SDSP	SYSP	PRO	FN	SED
Yield	1[a]	**	**	ns	ns	ns	ns	Ns	**	**	**	Ns	**	**	***	ns	*	ns
TW	0.59	1	****	****	****	ns	*	****	*	****	****	*	***	****	****	ns	**	****
TKW	0.62	0.87	1	****	***	ns	**	***	**	****	****	*	****	****	*** *	*	***	**
Days to Heading	-0.40	-0.87	-0.72	1	****	ns	ns	****	ns	****	****	Ns	ns	****	****	ns	****	***
Days to Maturity	-0.38	-0.87	-0.68	0.95	1	ns	ns	****	ns	****	****	Ns	ns	****	****	ns	**	***
Seedling m-2	-0.20	0.15	0.02	-0.27	-0.33	1	ns	Ns	ns	ns	ns	Ns	ns	ns	Ns	ns	ns	ns
Spikes m-2	-0.06	-0.46	-0.61	0.36	0.32	0.07	1	Ns	*	ns	*	**	*	ns	*	ns	ns	ns
Lodging	0.32	0.80	0.66	-0.80	-0.83	0.29	-0.29	1	ns	****	****	*	*	****	****	ns	*	***
Spike Length	0.55	0.49	0.59	-0.24	-0.25	-0.27	-0.48	0.41	1	ns	**	Ns	****	**	**	ns	ns	ns
FHB	-0.55	-0.80	-0.72	0.78	0.74	-0.13	0.22	-0.85	-0.38	1	****	Ns	*	****	****	ns	**	***
Height	0.57	0.95	0.83	-0.90	-0.87	0.15	-0.44	0.87	0.52	-0.86	1	**	***	****	****	ns	***	****
HI	-0.13	-0.50	-0.50	0.38	0.32	-0.13	0.53	-0.44	-0.39	0.38	-0.53	1	ns	ns	Ns	ns	ns	*
Spikelet Spike-1	0.62	0.65	0.74	-0.38	-0.36	-0.15	-0.49	0.50	0.85	-0.49	0.65	-0.34	1	***	****	ns	*	ns
Seed Spike-1	0.55	0.78	0.72	-0.75	-0.74	0.15	-0.34	0.81	0.56	-0.76	0.87	-0.22	0.71	1	****	ns	****	*
Seed Yield Spike-1	0.64	0.88	0.87	-0.82	-0.80	0.07	-0.47	0.80	0.61	-0.80	0.94	-0.35	0.76	0.96	1	ns	****	**
Protein	-0.39	-0.26	-0.46	0.37	0.29	-0.17	0.14	-0.01	-0.07	0.15	-0.23	0.00	-0.28	-0.27	-0.36	1	***	ns
FN	-0.41	-0.61	-0.65	0.73	0.59	-0.08	0.38	-0.49	-0.33	0.58	-0.69	0.31	-0.49	-0.73	-0.77	0.63	1	ns
SED	0.27	0.81	0.55	-0.69	-0.69	0.10	-0.38	0.64	0.24	-0.64	0.77	-0.46	0.31	0.51	0.61	0.17	-0.32	1

Note: [a] Correlation of coefficient. *, **, ***, ****: significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$. The Pearson correlation coefficients that are shaded in grey are consider to be significant at $P < 0.05$. TW=test weight; TKW=thousand kernel weight; FHB=fusarium head blight; HI=harvest index; FM-Falling Number; SED=SDS Sedimentation

There were four principal components (PC) with eigenvalues greater than 1 (Table 3.9). The first PC, which accounted for 57.2% of the total variation, differentiated between the short and tall NILs (Figure 3.2). The second, third, and fourth PC accounted for 11.5%, 9.12%, and 6.79% of the total variation, respectively, and did not differentiate the short and tall NILs (Figure 3.2; Figure 3.3; Figure 3.4). The first PC had the most significant positive eigenvectors for test weight, 1000-kernel weight, lodging rating, height, seed number spike^{-1}, and seed yield spike^{-1}. It had the most significant negative values for days to heading and FHB rating (Table 3.9). This indicated that these traits are the most important for describing the NILs.

3.4 Discussion

3.4.1 Gibberellic acid sensitivity

Previous studies have categorized *Rht18* as sensitive to GA (Konzak 1988). In the control groups the short NIL seedling length measurements were significantly shorter than all of the other cultivars' measurements, but in the GA treated group the measurements were the same as CDC Utmost and the tall NILs. Yang et al. (2017) reported that when GA is applied to lines carrying *Rht18,* the coleoptiles, and in some cases mature plant heights, are able to grow to similar lengths as the parents not carrying the allele.

It would be expected that wheat seedlings carrying *Rht18* would have shown a significant increase in growth when GA is applied. The semi-dwarf stature of wheat carrying this gene has been found to be due to a mutation in the region coding for the gene *GA2oxA9,* which encodes a GA 2-oxidase that converts an active GA intermediate into an inactive GA metabolite (Ford et al. 2018). The mutation results in an increase in expression of the *GA2oxA9* gene, which results in a decrease in bioactive GA production in the wheat. Therefore, it was expected that an application of GA would result in a phenotype similar to the tall NILs or regular height parent CDC Utmost.

The short and tall NILs both showed a significant response to the application of GA_3. In other studies on *Rht18* the corresponding tall parent also had a significant increase in coleoptile length, and in some cases in mature plant height, when GA was applied (Yang et al. 2017). CDC Utmost did show an increase in seedling length in response to GA application, but it was not statistically significant. The tall NILs did show a significant increase, but not as large a response as the short NILs. Other studies had similar results, where either the tall parental cultivars or tall NILs were responsive to GA application. In one study by Chen et al. (2014) the application of

Table 3.9. The eigenvectors of the first four principal component (PC) for 18 agronomic, plant structure, or quality variables for the 24 NILs. The first four PC had eigenvalues above 1.

Variable	PC1	PC2	PC3	PC4
Grain Yield	0.19	**0.30**	-0.20	**0.34**
TW	**0.30**	-0.07	0.06	0.03
TKW	**0.28**	0.14	-0.02	-0.15
Days to Heading	**-0.28**	0.24	0.11	0.04
Days to Maturity	-0.27	**0.28**	0.07	-0.03
Seedling m^{-2}	0.04	**-0.47**	-0.17	-0.26
Spike m^{-2}	-0.16	-0.18	-0.28	**0.55**
Lodging	**0.26**	-0.22	0.13	0.15
Spike Length	0.18	**0.45**	0.20	0.05
FHB	**-0.27**	0.12	-0.02	-0.24
Height	**0.31**	-0.07	0.06	0.06
HI	-0.16	0.01	-0.38	**0.43**
Spikelet $Spike^{-1}$	0.23	**0.40**	0.04	0.02
Seed $Spike^{-1}$	**0.28**	0.02	-0.11	0.17
Seed Yield $Spike^{-1}$	**0.30**	0.07	-0.08	0.07
Protein	-0.10	-0.13	**0.63**	0.35
FN	-0.23	-0.04	**0.32**	0.22
SED	0.22	-0.23	**0.33**	0.10
Eigenvalue	10.30	2.07	1.64	1.22
Proportion (%)	57.20	11.50	9.12	6.79
Cumulative (%)	57.20	68.70	77.82	84.61

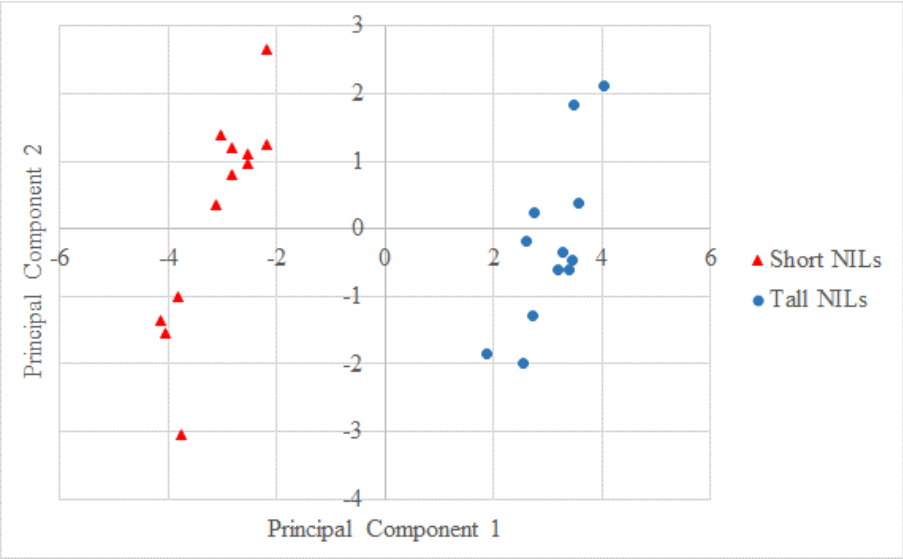

Figure 3.2. Scatter plot comparing principal component 1 and principal component 2 based on principal component analysis of 18 agronomic, plant structure, and quality traits of 24 NILs. The NILs are classified as short (red triangles) or tall (blue circles).

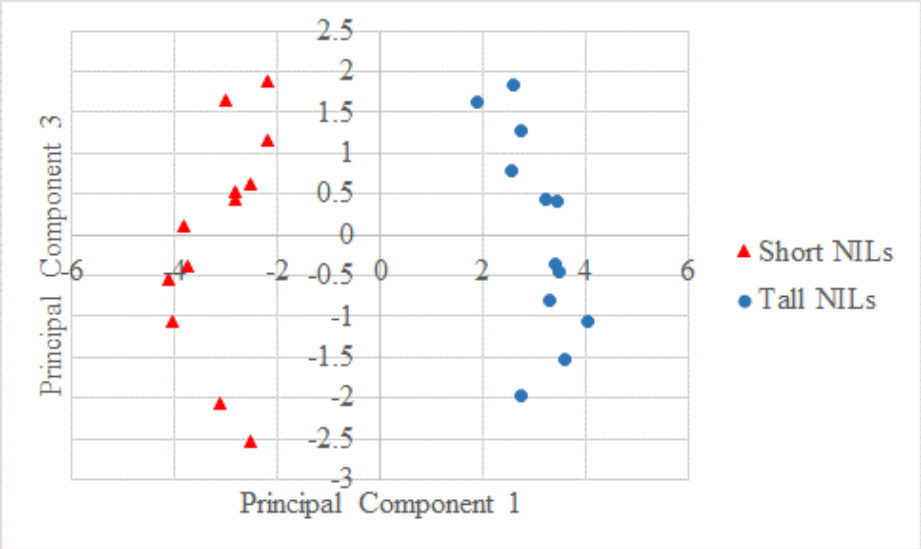

Figure 3.3. Scatter plot comparing principal component 1 and principal component 3 based on principal component analysis of 18 agronomic, plant structure, and quality traits of 24 NILs. The NILs are classified as short (red triangles) or tall (blue circles).

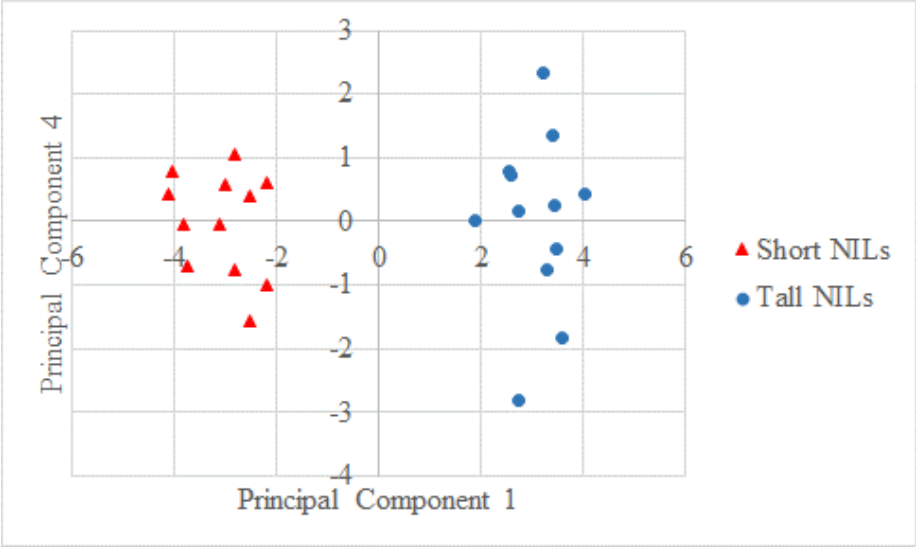

Figure 3.4. Scatter plot comparing principal component 1 and principal component 4 based on principal component analysis of 18 agronomic, plant structure, and quality traits of 24 NILs. The NILs are classified as short (red triangles) or tall (blue circles).

GA increased the coleoptile length, leaf area, and final plant height of both the *Rht12* semi-dwarf lines and the tall lines compared to the controls, but with less significance in the tall lines. Ellis et al. (2004) described how semi-dwarf genotypes could be classified into three categories. In the first, the semi-dwarf genotypes had the same response to GA as their corresponding tall lines, in the second the semi-dwarf genotypes did not respond to GA application even when their corresponding tall lines did, and in the third the semi-dwarf genotypes and corresponding tall

lines responded to GA application, but the semi-dwarf genotypes had a significantly greater response. *Rht18* fell into the third category, whereas GA insensitive genes *Rht-B1b* and *Rht-D1b*, fell into the second. Carberry carries the semi-dwarfing allele *Rht-B1b*, which is considered to be GA insensitive. In one study by Pandey et al. (2015), however, Carberry was partially sensitive to GA, which was confirmed by this study's results.

3.4.2 Coleoptile and root length measurements

The length of the coleoptile of a plant is important because when wheat is sown deep to take advantage of deeper soil moisture the coleoptile must become long enough to ensure the plant has fully emerged from the soil. Wheat cultivars carrying either *Rht-B1b* or *Rht-D1b* had a reduced emergence speed and frequency when seeded deeper due to their reduced coleoptile length (Schillinger et al. 1998). It was important to confirm whether wheat carrying *Rht18* would have the same problem.

The short and tall NILs had a similar primary and total root length, but the short NILs had a reduced coleoptile length in the current study. Other studies have reported varying results for the effect of *Rht18* on coleoptile and root growth. The durum cultivar Icaro, which was the *Rht18* donor parent in this study, had a significant reduction in coleoptile growth compared to its non-dwarf parent Anhinga (Ellis et al. 2004). The current study did not measure the coleoptile length of Icaro, so it was not possible to compare the reduction in coleoptile length in the short NILs relative to Icaro.

Another study by Yang et al. (2015) found that when combined with *Rht8*, *Rht18* winter wheat had a reduction in coleoptile length but an increase in root vigor. They also reported that with *Rht18* alone, there was no difference in coleoptile length or root characteristics in two semi-dwarf winter wheat populations compared to their parents. Similar results were reported, with no difference in coleoptile length between the regular height parents and two sets of durum RIL or between tall lines that did not carry *Rht18* and short lines that did carry *Rht18* in both bread wheat and durum wheat (Tang 2015).

Gibberellic acid sensitive wheat cultivars are generally considered to have longer coleoptiles compared to GA insensitive cultivars, and therefore are thought be a better option in drier areas where seed has to be sown deeper, and there is a greater potential for reduction in seedling emergence (Rebetzke et al. 1999). Gibberellic acid insensitive semi-dwarf plants have a reduction in cell size, even when endogenous GA is present. This results in a decrease in overall

all plant height, but also in coleoptile length. This can result in reduced emergence rate and seedling vigor under less ideal environment conditions (Richards 1992b). Some GA sensitive semi-dwarfing genes produced reduced coleoptile lengths as well, including *Rht11* and *Rht17* in hexaploid wheat and *Rht16* and *Rht18* in durum wheat (Ellis et al. 2004), similar to the current study. Therefore, it was important to measure the effect of individual semi-dwarfing genes in a different genetic backgrounds on coleoptile length and seedling emergence.

The short NILs RhtNIL 14015 and RhtNIL 14021 had coleoptile lengths statistically similar to the tall NILs and CDC Utmost. This shows that even though the short NILs had, on average, a shorter coleoptile length, it was possible to select a short NIL with a semi-dwarf stature and a longer coleoptile length if plants needed to be sown deeper and reduced seedling emergence was a potential concern in some environments.

3.4.3 In-field plant development measurements

The short NILs carrying *Rht18* headed and matured approximately two days later than the tall NILs. In one study by Tang (2015), lines carrying *Rht18* and *Rht-D1b* flowered at the same time as the corresponding tall lines, and therefore neither of these semi-dwarfing genes had effect on development time. It is possible that the rate of plant development in wheat carrying *Rht18* was delayed compared to other genetically similar lines that did not carry the gene, but this can also vary depending on the genetic background and environment that the plant is grown in. Wheat and other crops grown in lower latitude areas, such as Australia, would have a different physiological development timeline compared to in higher latitudes such as in Canada. Time to flowering and the rate of other physiological developments is affected by the differences in the vernalization, photoperiod, and temperatures, which can vary greatly between environments (McMaster et al. 2008). Therefore, it is difficult to compare the relative time for plant development in wheat in different areas of the world.

Seedling emergence is a concern when growing semi-dwarf wheat cultivars carrying either of the dwarfing alleles *Rht-B1b* or *Rht-D1b* as they may exhibit a reduction in plant emergence (Schillinger et al. 1998). Even though in the presence of the *Rht18* semi-dwarfing allele the short NILs had a reduction in coleoptile length, there was no reduction in seedling emergence as measured at the 3-leaf stage compared to the tall NILs. Tang (2015) reported similar results. When sown in outdoor trays there was no significant difference in emergence between *Rht18*-carrying and *Rht18*-not carrying lines when sown at different depths (3 cm, 9 cm,

or 12 cm). They found that at the 9 cm seeding depth the *Rht18* and tall lines had similar emergence, whereas the *Rht-D1b* and double dwarf lines had a reduced emergence.

There was no difference in emergence between any of the NILs or checks in the present study. The majority of short NILs had a similar coleoptile length compared to the two *Rht-B1b* cultivars, Carberry and CDC Go, which were found to be significantly shorter than the tall NILs and CDC Utmost. It is possible that if sown deeper, the short NILs would not have had the same emergence as the tall NILs, but this issue was not observed in the current study. It has also been reported that coleoptile length does not have a linear relationship with seedling emergence, and other factors such as genetic background and environmental conditions can effect emergence (Mohan et al. 2013). Further studies would have to be conducted to determine whether the short *Rht18* NILs would have a reduced emergence if grown in environments that require deeper sowing depths.

There was no reduction in the number of mature spikes per unit area for short NILS compared to the recurrent parent and a 3.0% increase relative to the tall NILs. These results matched with another study, where there was no difference in the number of spikes m^{-2} between *Rht18* semi-dwarf and tall bread wheat lines (Tang 2015).

Lodging can complicate harvest, increase susceptibility to diseases and pests, and negatively impact crop development by decreasing grain m^{-2} and average grain weight (Piñera-Chavez et al. 2016a). Semi-dwarf plants are known to reduce stem lodging due to their reduced height (Gale and Youssefian 1985). The short NILs showed no lodging, an improvement over both CDC Utmost and the tall NILs. In the 2016 and 2017, the growing conditions were not favorable to lodging, so lodging was not observed at every location. In years where a high potential for lodging is present, *Rht18* carrying lines could have a potentially large benefit for wheat production.

There are typically two forms of lodging found in cereal crops, stem and root lodging. Stem lodging can be split into two types, which include culm bending and culm breaking. The reduction in stem lodging by culm bending in semi-dwarf plants is in part due to reduction in height. Therefore, the semi-dwarf lines would be expected to have less lodging by culm bending than the tall NILs and tall parent (Hirano et al. 2017). Lodging due to culm breaking has a variety of different causes besides plant height, including the morphology of the plant as well as the quality of the stem components. In rice, semi-dwarf lines were found to have a reduction in

stem diameter as well as reduction in lignin content, which are both important components to reduce lodging caused by stem breakage. These reductions are believed to be due to reduced GA content in the plant (Okuno et al. 2014). Chen et al. (2014) found that when wheat lines carrying *Rht12* had GA applied to them they had increased internode lengths and height, as well as a decrease in stem diameter and wall thickness to levels even shorter and thinner than the corresponding tall NILs. These lines had increased lodging compared to the *Rht12* controls where no GA was applied, with lodging similar to the tall lines. The morphology of wheat plants, whether carrying a semi-dwarf gene or not, appears to depend on more than just the presence or absence of a single gene. Stem diameter, wall thickness, and lignin content would have to be measured to ensure that wheat lines carrying *Rht18* has good resistance to lodging caused by stem breakage, as well as due to a reduction in plant height. Root lodging is generally caused by environmental conditions such as poor soil or rainfall or by poor development of root anchorage by the plant (Berry et al. 2003). In the current study, the short NILs had similar primary and total root lengths relative to the tall NILs at the plumule emergence stage. Further studies would have to be conducted to test whether there is any difference in root lodging resistance between short and tall NILs.

The short NILs had a higher FHB infection level than the tall NILs, which were similar to CDC Utmost. The short NILs had a similar infection level to CDC Go. There are five types of FHB resistance in wheat (Mesterhazy et al. 1999). Of the five types, the first two types of resistance, Type I - avoidance to initial infection and Type - II resistance to spread within the spike of infected tissue, have been studied in semi-dwarf wheat. To date, there have been no reports of the effect of *Rht18* in wheat on FHB response.

Type I resistance was found to be greater in 10 tall NILs, and highly significantly so, for eight tall NILs, compared to their semi-dwarf NIL counterparts for the genes *Rht-B1b*, *Rht-D1b*, *Rht-B1c*, *Rht4* (two difference populations), *Rht5*, *Rht8*, *Rht8+Rht9*, *Rht11*, and *Rht13* at their natural heights (Yan et al. 2011). This difference in avoidance was not present for nine of the 10 genes when the semi-dwarf plants were artificially raised to the same height as the tall NILs, showing that decrease in initial infection was largely due to the physical height difference, not genetic difference, in these semi-dwarf wheats. Type II resistance levels varied between genotypes. Only the four genes Rht*4*, *Rht8 + 9*, *Rht-B1b*, and *Rht-B1c* showed a significant difference, and in all four cases the semi-dwarfing NILs had increased resistance to FHB spread

over the tall NILs (Yan et al. 2011).

In the current study the cultivar Carberry, which carried the semi-dwarfing gene *Rht-B1b* and was significantly shorter than the tall NILs and CDC Utmost, had significantly less FHB infection, indicating other physiological traits and background genetics affected FHB susceptibility and reduced the negative effects the semi-dwarfing genes may have. QTL for FHB resistance have been reported on chromosome 6A in both tetraploid and hexaploid wheat, with one being found near the centromere on the long arm of chromosome 6A in winter wheat (Schmolke et al. 2005) and one found on the short arm of chromosome 6A in durum wheat (Prat 2016). Both of these QTL were found to have overlap with QTL for plant height located on chromosome 6A, with a negative correlation reported between plant height and FHB infection. Genetic analysis for QTL for FHB resistance was not analyzed in the current study, therefore it was unknown whether the increased susceptibility to FHB in the short NILs was due to the shortening of the plant, genetic linkage of *Rht18* with QTL, or other possible pleiotropic effects. Further work would have to be performed to analyze the cause of the increased susceptibility of short NILs to FHB infection.

3.4.4 Plant height and internode lengths

The reduction in plant height in wheat carrying the *Rht-B1b* and *Rht-D1b* semi-dwarf genes is typically believed to be caused by a reduction in cell elongation, rather than cell division, due to decreased cell wall extensibility (Keyes et al. 1989). This is due to the cell's insensitivity to endogenous GA (Richards 1992b). Currently, the cause of reduced stem elongation and height for *Rht18* semi-dwarf plants is not known. *Rht18* has been found to have a mutation that results in increased production of a GA 2-oxidase, which results in a decrease in endogenous GA synthesis (Ford et al. 2018) Therefore, it is likely that this height reduction is caused by a GA deficiency, which would have symptoms similar to semi-dwarf plants that do not respond to GA.

The short NILs were 16 cm shorter (17.7% reduction) compared to the tall NILs. The reduction in height was due to a reduction in the length of all of the internodes as well as the spike length. Other studies reported varying results, with all the lines carrying *Rht18* having a reduced height compared to their corresponding tall lines, but different effects of the gene on the individual spike and internode lengths and degree of height reduction. Yang et al. (2015) reported a 25.0%, 12.4%, and 15.5% reduction in three different $F_{4:5}$ winter wheat populations

carrying *Rht18* (the third one also carrying *Rht8*) compared to their regular height parents. There was a 25% reduction in height for an *Rht18* Australian bread wheat population when compared to corresponding tall lines (Tang 2015).

In one study *Rht18* reduced height significantly over the tall lines with the majority of reduction in height resulting from a reduction in the length of the top three internodes (Tang 2015). Yang et al. (2015) reported differing results in two separate $F_{4:5}$ Chinese winter wheat populations carrying *Rht18*, with one having a reduction in length in the top three internodes and one having a reduction in length in all five internodes. In the current study, the short NILs had a reduction in all of the internodes, but the majority of reduction in height was due to the top internodes, as depicted in Figure 3.1.

Along with the reduction compared to tall NILs, there was also an 8 cm reduction in height compared to the *Rht-B1b* semi-dwarf cultivars Carberry and CDC Go. This reduction in height in the short NILs compared to *Rht-B1b* semi-dwarf cultivars was not reported by Tang (2015). In their study, lines carrying *Rht18* had a similar height and internode lengths compared to plants carrying *Rht-B1b* or *Rht-D1b*.

In a previous study, there was no difference between mature spike lengths between either the *Rht18*, *Rht-D1b*, or tall lines (Tang 2015). Another study reported a slight increase in spike length of approximately 1.4 cm and 0.9 cm in two semi-dwarf populations carrying *Rht18* compared to their regular height parents (Yang et al. 2015), which were confirmed in a similar study in the same populations (Yang et al. 2017). In the current study, a difference of less than 1 mm in spike length was observed between the short and tall NILs, as well as overlap between individual lines within each NIL group.

3.4.5 Agronomic traits

The short NILs had a reduction in grain yield, test weight, thousand kernel weight, number of spikelets spike^{-1}, number of seeds spike^{-1}, and seed yield spike^{-1} compared to the tall NILs. Taking the statistical analysis into account, the mean effect on test weight, thousand kernel weight, number of spikelets spike^{-1}, number of seeds spike^{-1}, and seed yield spike^{-1} were relatively small, with a decrease for the short NILs compared to the tall NILs of 1.7%, 3.0%, 2.0%, 9.3%, and 12.4%, respectively.

The short NILs yielded significantly less than the tall NILs. Variation between NIL groups for yield was observed, with some short NILs yielding higher than others with yields

similar to the tall NILs, as well as CWRS cultivars CDC Utmost and Glenn. Few studies have been conducted on *Rht18* in yield trials. One study measured yield in a replicated (r=2) trial at one location for one year and detected a slight, but non-significant increase in yield m^{-1} in the *Rht18* lines compared to the tall lines (Tang 2015). Most yield measurements in that study were conducted on a per row or per plant scale, which may not translate to a plot scale. No significant difference in yield was observed between *Rht18* genotypes and their tall parents (Yang et al. 2015; Yang et al. 2017).

The short NILs had a small, but statistically significant (P = 0.0002) increase in HI compared to the tall NILs. In Australian bread wheat lines carrying *Rht18,* there was an increased HI compared to the tall lines (Tang 2015). In the same study the *Rht18* semi-dwarf lines had the same HI as semi-dwarf *Rht-D1b/B1b* lines, whereas in the current study the *Rht-B1b* semi-dwarf cultivars Carberry and CDC Go both had a higher HI than the short NILs. Some of the individual short NILs had a similar HI to Carberry. Thus, when considering the potential of *Rht18,* it was important to look at individual lines to select the best possible combination of traits. In two studies by Yang et al. (2015; 2017) the *Rht18* lines had a slight increase in HI compared to their tall parents, similar to the current study, except this increase was only significant in one of the three winter wheat populations. Semi-dwarf plants tend to have a decrease in overall aboveground biomass, which would support the increase of HI, but *Rht18* lines have also been found to potentially have reduced seed yield traits, such as lower thousand kernel weight (Yang et al. 2017) and grain yield (Yang et al. 2015). Even though the semi-dwarf gene resulted in a reduction in biomass, a reduction in grain yield (kg ha^{-1}), thousand kernel weight, and seed yield $plant^{-1}$ curtailed the increase in HI in plants carrying *Rht18*.

In other studies, there appears to be an inverse relationship between grain number $spike^{-1}$ and thousand kernel weight (Yang et al. 2015; 2017). The opposite was observed in the current study with a significant positive correlation between the two traits. Even though the short NILs showed a reduction in the majority of agronomic traits compared to the tall NILs, they were within the normal range compared to other wheat cultivars that were measured in the current study (Appendix E).

3.4.6 Seed quality measurements

Since the main use of CWRS wheat is for bread production, it is important that the end-use quality is ideal for baking in semi-dwarf lines carrying *Rht18* in a CWRS background. The

protein level and quality is important for ensuring proper bread formation, and to be considered a CWRS wheat in Canada a certain quantity of protein is required relative to check cultivars. The short NILs had an overall statistically significant increase in protein percentage compared to the tall NILs, but there was significant variation within the NIL groups and plant height was not found to be statistically correlated with protein level.

The gene *NAM-A1* (also called *GPC-A1*) is believe to be involved in increased nutrient remobilization and an increased protein content in wheat (Avni et al. 2014). Cormier et al. 2015 located this gene on chromosome 6A at a location within 5 cM of the majority of the SNPs that were found to be polymorphic between the short and tall NILs (detailed in chapter 4). Bread wheat carrying *NAM-A1* had varying results for correlation of the gene with grain yield and thousand kernel weight. One report indicated that *NAM-A1* correlated with a reduction in grain yield due to a reduction in thousand kernel weight (Cormier et al. 2015) and another study reported no correlation (Avni et al. 2014). Therefore, it is not likely that a lower protein content in tall NILs were due to a dilution effect of the larger seed size (Avni et al. 2014).

In the current study there was a statistically significant negative correlation between protein level and thousand kernel weight, but not yield, for the NILs. It was possible that the higher protein and lower thousand kernel weight in the short NILs was a result of more bran in the seed, which is higher in protein than the endosperm, but this was not analyzed. It is uncertain whether the increase in protein was due to genetic factors in the current study, and the significant variation in grain protein levels and grain yield within NIL groups made it difficult to speculate a direct cause.

A higher FN is desired in bread wheat because it is generally associated with less alpha-amylase activity, and therefore less sprouting damage. A high level of alpha-amylase in bread dough results in degraded starch, which then results in sticky dough and a low quality bread loaf (Farrand 1964). GA is an important plant hormone that is involved in stimulating seed germination. In cereals, GA stimulates the production of alpha-amylase in the aleurone layer of the seed. Therefore, it is possible that seeds from semi-dwarf plants with lower levels of endogenous GA have a decrease in alpha-amylase activity (Sponsel and Hedden 2010). The GA insensitive gene *Rht-B1c* has been found to produce less enzyme activity due the aleurone layer being insensitive to GA (Gale and Marshall 1973). The *Rht-B1b* and *Rht-D1b* genes have also resulted in an increase in FN (Casebow et al. 2016) but have not been found to produce less

alpha-amylase in the aleurone in the presence of GA (Gale and Marshall 1973). Along with differences in GA biosynthesis and regulation, the FN value can also be influenced by other genetic and environmental factors.

When it rains or there is a humid environment that causes the wheat to be moist in the field or in storage, it is possible for the plant to begin germinating in the spike before it is harvested. This can result in an increased level of alpha-amylase (Gale and Marshall 1975). Therefore, even if a certain cultivar is genetically expected to be resistant to sprouting, adverse moisture conditions will result in a decrease in FN. The wheat samples in the current study were harvested and stored using the same methods as one another so it would be less likely, but possible that difference in FN was due to external environmental conditions. Wheat cultivars that contains waxy (amylose-free) starches have also been found to have a reduced FN due to a reduction in viscosity even when the level of the alpha-amylase enzyme activity is low (Eliasson 2012). This demonstrated that even though the FN test is useful in screening wheat seed for alpha-amylase activity, it is not an exact measurement of enzyme activity.

The SDS sedimentation test screens wheat flour samples for their relative gluten strength based on the quality and quantity of protein (AACC International 1999c). A high SDS sedimentation volume is desired to ensure a certain level of protein quality. The tall NILs had significantly higher SDS sedimentation volumes than the short NILs. There was a marker trait association for flour SDS sedimentation detected on chromosome 6A in wheat, but it had a small phenotypic effect (Jernigan et al. 2018). Major genes on chromosome 6A and chromosome 6B regulate alpha-gliadin and beta-gliadins, which are soluble protein fractions of gluten, in both durum and hexaploid wheat (Wrigley and Shepherd 1973; duCros et al. 1983). It has been demonstrated that SDS sedimentation volume and gluten strength is affected more by the glutenin fraction than the gliadin fraction, but a decrease in glutenin:gliadin ratio can result in a decreased mixing time, mixograph peak resistance, maximum resistance to extension, and loaf volume and an increased resistance breakdown and extensibility (Uthayakumaran et al. 1999; Pistón et al. 2011). Even though there was statistically significant difference found between the quality characteristics of the short NILs and tall NILs, in the current study the mean differences were small. Baking tests would have to be conducted to determine effects of *Rht18* on bread quality.

3.5 Conclusions

Rht18 carriers were sensitive to the external application of the plant hormone GA and therefore could be classified as GA sensitive. Short NILs carrying *Rht18* had a reduced coleoptile length compared to the recurrent parent CDC Utmost and tall NILs but showed no relative reduction in root length.

The short NILs did not have reduced seedling emergence or spikes per unit area compared to CDC Utmost or the tall NILs, but did have an increase in days to heading and days to maturity of approximately 2.4 and 2.3 days respectively, compared to the tall NILs. The short NILs also had a significant reduction in lodging compared to the tall NILs. There was a significant reduction in height in the short NILs compared to the tall NILs, and this was due to a reduction in spike length and a reduction in the length of all the internodes.

The short NILs were lower in yield (2.4% decrease) and had a higher HI (2.1% increase) compared to the tall NILs. There was also a small, but statistically significant reduction in test weight, thousand kernel weight, spikelets spike^{-1}, seeds spike^{-1}, and seed yield spike^{-1} for the short NILs relative to the tall NILs. The short NILs had a slightly higher grain protein content and FN, but slightly lower SDS Sedimentation volume compared to the tall NILs.

4.0 Development of a KASP marker for the *Rht18* semi-dwarfing gene in hexaploid wheat (*Triticum aestivum* L.)

4.1 Introduction

Semi-dwarf wheat cultivars have been used widely throughout the world, specifically starting in the early 20th and mid-20th centuries (Gale and Youssefian 1985; Hedden 2003). Traditionally, little was known about the specific genes inducing reduced height and screening for a dwarfing gene consisted of growing the wheat plants to full maturity and manually measuring the height. Using molecular markers during the selection process is ideal because it allows for indirect selection of a plant early in the life cycle, such as the seedling stage, for a specific trait that might not be measured until later in the life cycle. *Rht-B1b* and *Rht-D1b* are two of the most common semi-dwarfing genes used in wheat breeding and cultivar development, and have robust molecular markers developed to aid in selection of these genes (Ellis et al. 2002). Identifying a molecular marker linked to the *Rht18* gene would help increase the rate of selection for semi-dwarf plants that specifically carry this gene.

The *Rht18* gene has been mapped in multiple durum wheat populations to the short arm of chromosome 6A, and some potential molecular markers were identified, including the SSR marker *S470865SSR4* (Haque et al. 20l1; Vikhe et al. 2017). SNP markers, and specifically KASP assays, are popular due to their ease of use and low cost (Semagn et al. 2014). Codominant markers are ideal because they distinguish between both homozygotes and heterozygotes genotypes. KASP genotyping assays are the type of SNP markers presently used in wheat breeding at the University of Saskatchewan and therefore it is desirable to identify a molecular marker for the *Rht18* gene that can be used in a KASP assay.

4.1.1 Research Hypothesis and Objectives

The hypothesis for chapter 4 was that a KASP molecular marker for the *Rht18* gene could be developed and used to differentiate between the semi-dwarf spring wheat lines that carried the *Rht18* gene in both heterozygous and homozygous states, and regular height spring wheat lines.

To achieve this objective a KASP molecular marker for the *Rht18* gene had to be developed that could distinguish between CDC Utmost plus the 12 normal height NILs and Icaro

plus the 12 semi-dwarf NILs. This marker was validated on a segregating F_2 population. The marker was required to determine whether the gene was in a homozygous or heterozygous condition, as well as differentiate *Rht18* carriers from lines carrying other dwarfing genes as well as regular height plants.

4.2 Materials and Methods

4.2.1 Plant materials

Icaro is a dwarf durum wheat cultivar, the donor of the *Rht18* gene, which was obtained from fast-neutron induced mutation of the durum cultivar Anhinga (Konzak 1988). Icaro was crossed with the hexaploid wheat cultivar CDC Utmost and subsequently backcrossed with CDC Utmost for five cycles (CDC Utmost*6/Icaro), developing a set of NILs. These lines were developed at the CDC in Saskatoon, Saskatchewan by Pierre Hucl. A total of 24 NILs were used for primer design and KASP marker development. Of these 24 lines, 12 lines were classified as tall and 12 lines were classified as short-strawed, with the 12 short lines carrying *Rht18*. The parental cultivars CDC Utmost and Icaro, as well as the three check cultivars Carberry, Glenn, and CDC Go were used throughout the project. The short lines included RhtNIL 14005, RhtNIL 14015, RhtNIL 14016, RhtNIL 14019, RhtNIL 14020, RhtNIL 14021, RhtNIL 14022, RhtNIL 14023, RhtNIL 14024, RhtNIL 14027, RhtNIL 14028, and RhtNIL 14029. The tall lines included RhtNIL 14003, RhtNIL 14004, RhtNIL 14006, RhtNIL 14007, RhtNIL 14010, RhtNIL 14011, RhtNIL 14012, RhtNIL 14013, RhtNIL 14014, RhtNIL 14017, RhtNIL 14018, and RhtNIL 14032.

4.2.2 DNA extraction and 90K SNP chip array

DNA was extracted from new leaf growth at the fourth-leaf seedling stage for the 24 NILs and five checks using the CTAB DNA extraction procedure for grasses, which was a modified version of Procunier et al. (1990). The quality of the DNA was checked visually using a 1.5% agarose gel made in 1x TBE buffer containing ethidium bromide at approximately 130 V for 1 hour. The DNA was visualized using a UV lamp.

The DNA for the 29 lines was sent to Agriculture and Agri-Food in Saskatoon where it was analyzed using a 90k SNP array chip following the Infinium® HD Assay Ultra Protocol Guide (Illumina Inc. San Diego, California, United States). The SNP data was analyzed using GenomeStudio Software (Illumina, San Diego, CA, USA). The SNPs that were polymorphic between the regular height wheat (12 tall lines and CDC Utmost), and the semi-dwarfed wheat

(12 short lines and Icaro) were nearly all (128 out of 131 SNPs) found on chromosome 6A. Fifty-four of the SNPs were used in the KASP marker tests.

4.2.3 KASP marker primer development

Forward and reverse KASP primers were designed for the 54 SNPs and ordered from Invitrogen™ (Thermo Fisher Scientific Inc., Waltham, Massachusetts, United States). For each of the SNPs there were three primers, two allele specific primers, and one common reverse primer. One of the forward primers was labelled with a HEX™ dye and the other was labelled with a FAM™ dye. Each of the three primers per SNP analysis was diluted to a 0.1mM DNA solution using 10mM Tris HCl. Primer stocks were made for each KASP marker by adding 12 µL of HEX™-labelled forward primer, 12 µL FAM™ labelled forward primer, 30 µL common reverse primer, and 46 µL ddH2O to a tube. The KASP reaction master mix (1x reaction) consisted of 0.11 µL primer stock, 4 µL KASP Master Mix (containing the universal FRET cassettes, ROX™ passive reference dye, Taq polymerase, free nucleotides, and $MgCl_2$), 2.89 µL sterilized H_2O, and 1 µL (50 ng) sample DNA, with a total volume of 8µL. The temperature cycling for the KASP reaction included 21°C for 10 s, 94°C for 15 min, 10 cycles of 94°C for 20 s and 57°C for 1 min, 35 cycles of 94°C for 20 s and 57°C for 1 min, 21°C for 3 min, three cycles of 94°C for 20 s and 57°C for 1 min, 21°C for 3 min, three cycles of 94°C for 20 s and 57°C for 1 min, 21°C for 3 min, three cycles of 94°C for 20 s and 57°C for 1 min, and finally 21°C for 3 min. The 54 KASP markers were run on the original DNA extracted from the 24 NILs and 5 check cultivars. The SNP data from the KASP runs were manually called and the markers that showed good separation clusters between the regular height and semi-dwarf wheat lines were selected for further testing.

4.2.4 F_2 mapping population

For validation, a F_2 population was developed by crossing one of the semi-dwarf NILs and one of the regular height NILs (RhtNIL 14029/RhtNIL 14012). These two NILs were selected as the lines most genetically similar to CDC Utmost, but with one carrying the *Rht18* gene and one not. This was analyzed visually by creating a dendrogram and by calculating the dissimilarity matrix of the 24 NILs relative to CDC Utmost using the original 90k SNP array chip data. Analysis was performed using DARwin software version 6 (Perrier and Jacquemoud-Collet, 2006), with the dissimilarity index calculated by Simple Matching Dissimilarity Index (Sokal-Michener Index) with 10000 bootstrap iterations (Perrier et al. 2003). The dendrogram

was clustered hierarchically using the Unweighted Neighbor-Joining method (Gascuel, 1997).

F_1 seed from the crosses were harvested and subsequently sown. F_2 seed was harvested and 600 seeds were sown, along with RhtNIL 14012, RHTNIL 14029, CDC Utmost, and Icaro as checks, with 3 seeds sown per pot. DNA from these 600 F_2 plants and check plants were extracted using the same methods described previously. The plants were grown to maturity in a controlled environment chamber at 21ºC for 18 hour days and 18ºC at night. The heights of the matured plants were measured using a ruler, measuring from the stem base to the top of the spike.

4.2.5 Validation of KASP markers

Validation was conducted on the DNA, extracted from the 600 F_2 plants and check cultivars, using the KASP methods described previously. The eight KASP markers selected for validation included IWB9704, IWB29660, IWB39455, IWB44856, IWB52347, IWB52666, IWA1423, and IWA1813. These markers were selected because they showed well separated clusters between standard height and semi-dwarf wheat lines and they were located on chromosome 6A.

The SNP data was manually called into three categories, with three separate clusters formed for regular height homozygotes, semi-dwarf homozygotes, and heterozygotes. The marker calls were visually compared to the phenotypic height data of the mature plants. Based on the results, the phenotypic data was grouped into three categories. Plants were considered semi-dwarf plants if the height was less than 720 mm and considered tall if the height was more than 780 mm. There was an overlap for semi-dwarf and tall F_2 plants between 720 and 780 mm where SNPs called as homozygous semi-dwarf and homozygous tall both occurred.

The same KASP markers were also evaluated on a total of 125 different cultivars not known to carry the *Rht18* allele to check their validity on a range of wheat cultivars. The cultivars consisted of 95 hexaploid wheat, 24 durum, one triticale (x *Triticosecale* Wittmack), one emmer (*Triticum turgidum* spp. *dicoccum*), one khorasan wheat (*Triticum turanicum* Jakubz.), two spelt (*Triticum spelta* L.), and one barley (*Hordeum vulgare* L.) accession.

4.2.6 Phenotypic selection of Rht18 carriers using the distance between the flag leaf and basal spikelet node

While growing out the F_2 plants for the genetic marker validation, it was noticed that semi-dwarf plants appeared to have a shorter length from the flag leaf to the base of the spike.

For this reason, during height measurement this distance was measured. Using a ruler, the length of the distance from the ligule of the flag leaf to the basal node of the spike was measured. For some plants the wheat spike was not fully emerged at maturity and therefore the distance was measured as a negative number. The statistical analysis was performed using SAS® version 9.4 (SAS Institute, Inc. Cary, NC, USA). The Pearson's correlation coefficient was calculated using the PROC CORR function and the linear regression was calculated using the PROC REG function.

4.3 Results

4.3.1 90k SNP Analysis

Of the 90000 SNPs in the chip array, just under 79000 were successfully read. These SNPs were used to construct a dendrogram visually comparing the 24 NILs and the five checks (Figure 4.1). Two main clusters formed separating the NILs into two groups, shown as separate branches, with CDC Utmost clustering in the branch with the tall NILs. The tall NIL most genetically similar to CDC Utmost was RhtNIL 14012 and the short NIL which was most genetically similar to CDC Utmost was RhtNIL14029. This information was confirmed by calculating the dissimilarity matrix of each of the 24 NILs compared with CDC Utmost (Table 4.1). RhtNIL 14029 had a dissimilarity of 0.17739 and RhtNIL 14012 had a dissimilarity of 0.01657 compared to CDC Utmost.

4.3.2 F_2 mapping population

Of the 600 F_2 plants that were grown for validation, 588 plant both grew to maturity and successfully had DNA extracted from them. When the phenotypic height data was assigned to the corresponding called SNP data, there was a bell curve shape for each of the homozygous tall homozygous semi-dwarf, and heterozygous plants (Figure 4.2). The heterozygous plants had a height ranging from 500 to 840 mm, which overlapped both semi-dwarf and tall plant distributions, which was expected due to the semi-dominant nature of *Rht18*. This was a similar range for the two parents of the F_2 plants with the minimum for RhtNIL 14029 being 497 mm and the maximum for RhtNIL 14012 being 868 mm. A chi-square test for the F_2 population ($\chi 2$ = 2.3776, $P > 0.05$) indicated that the expected segregation ratio of 1:2:1 failed to be rejected, indicating that the F_2 population of 150 tall, 307 heterozygous, and 131 semi-dwarf F_2 plants fit the Mendelian model of inheritance.

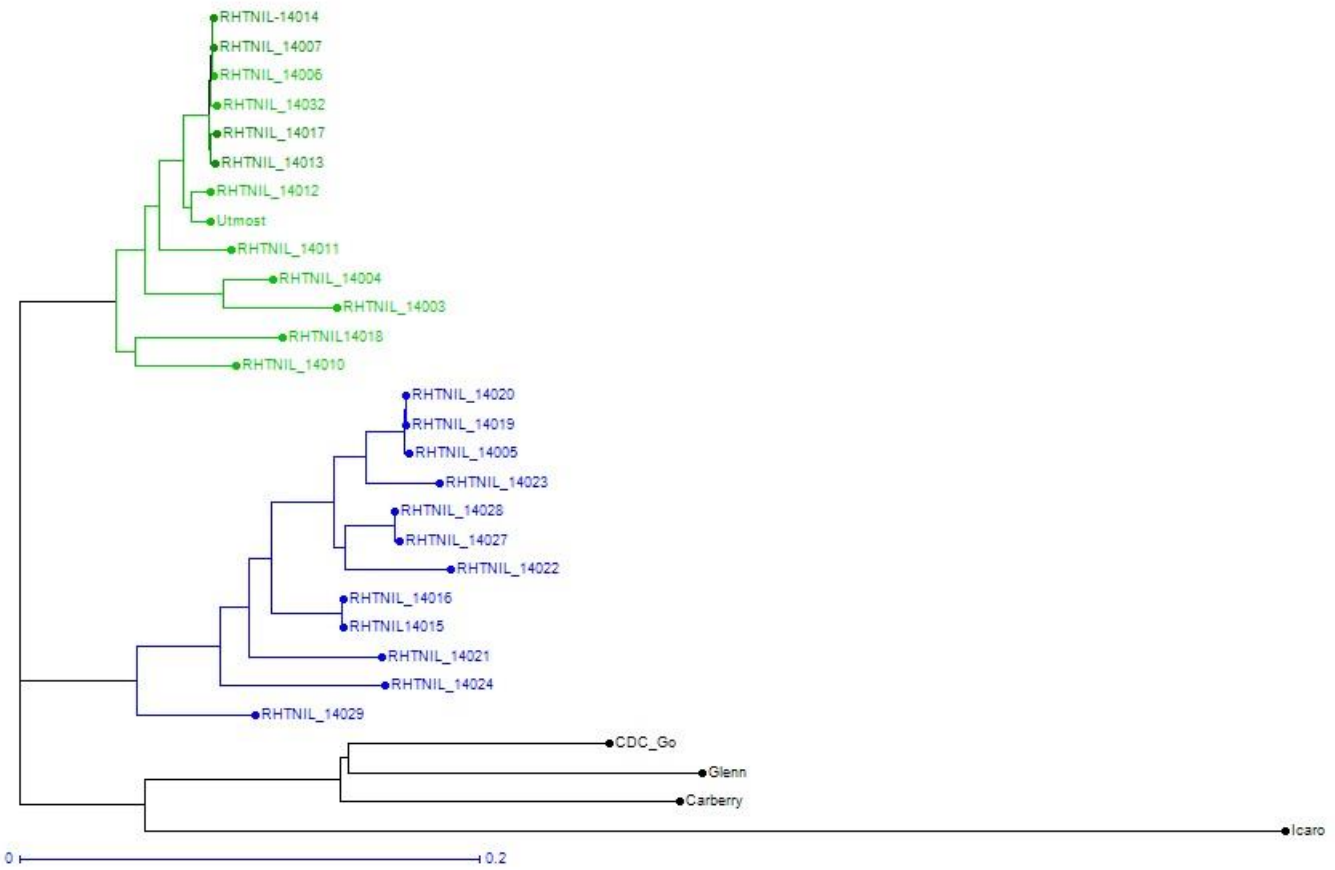

Figure 4.1. Dendrogram of the 24 near-isogenic lines and five checks hierarchically clustered. The green cluster included the tall NILs and CDC Utmost. The blue cluster included the short NILs. Clustering was conducted using the Unweighted Neighbor-Joining method in DARwin software version 6. The analysis was conducted based on the 90 k SNP data for each independent sample.

Table 4.1. The dissimilarity matrix of the 24 near-isogenic lines and Icaro compared to CDC Utmost. A smaller dissimilarity matrix number indicates more similarity to CDC Utmost. The two NILs most similar to CDC Utmost for each NIL group were chosen as the parents from the F_2 population. The dissimilarity index was calculated using 90k SNP data by Simple Matching Dissimilarity Index (Sokal-Michener Index) with 10000 bootstrap iterations in DARwin software version 6.

Lines	Dissimilarity Matrix
Tall NILs	
RhtNIL 14012	0.01657
RhtNIL 14006	0.02067
RhtNIL 14007	0.02067
RhtNIL 14014	0.02067
RhtNIL 14013	0.02312
RhtNIL 14032	0.02394
RhtNIL 14017	0.02475
RhtNIL 14004	0.06467
RhtNIL 14011	0.06702
RhtNIL 14010	0.10151
RhtNIL 14003	0.10451
RhtNIL 14018	0.11644
Short NILs	
RhtNIL 14029	0.17739
RhtNIL 14015	0.20808
RhtNIL 14016	0.20808
RhtNIL 14021	0.22994
RhtNIL 14024	0.23776
RhtNIL 14028	0.25510
RhtNIL 14027	0.25701
RhtNIL 14019	0.25891
RhtNIL 14020	0.25891
RhtNIL 14005	0.26081
RhtNIL 14023	0.27459
RhtNIL 14022	0.27770
Icaro	0.72252

4.3.3 Validation of Markers

Each of the markers had varying degrees of grouping tightness, but called each F_2 plant the same. Three distinct clusters were formed, categorizing the F_2 plants as either homozygous tall, homozygous semi-dwarf, or heterozygous (Figure 4.3). CDC Utmost, RhtNIL 14012, and the F_2 plants called as tall, clustered along the y-axis, with a high HEX fluorescence value and low FAM florescence value. Icaro, RhtNIL 14029, and the F_2 plants called as semi-dwarf,

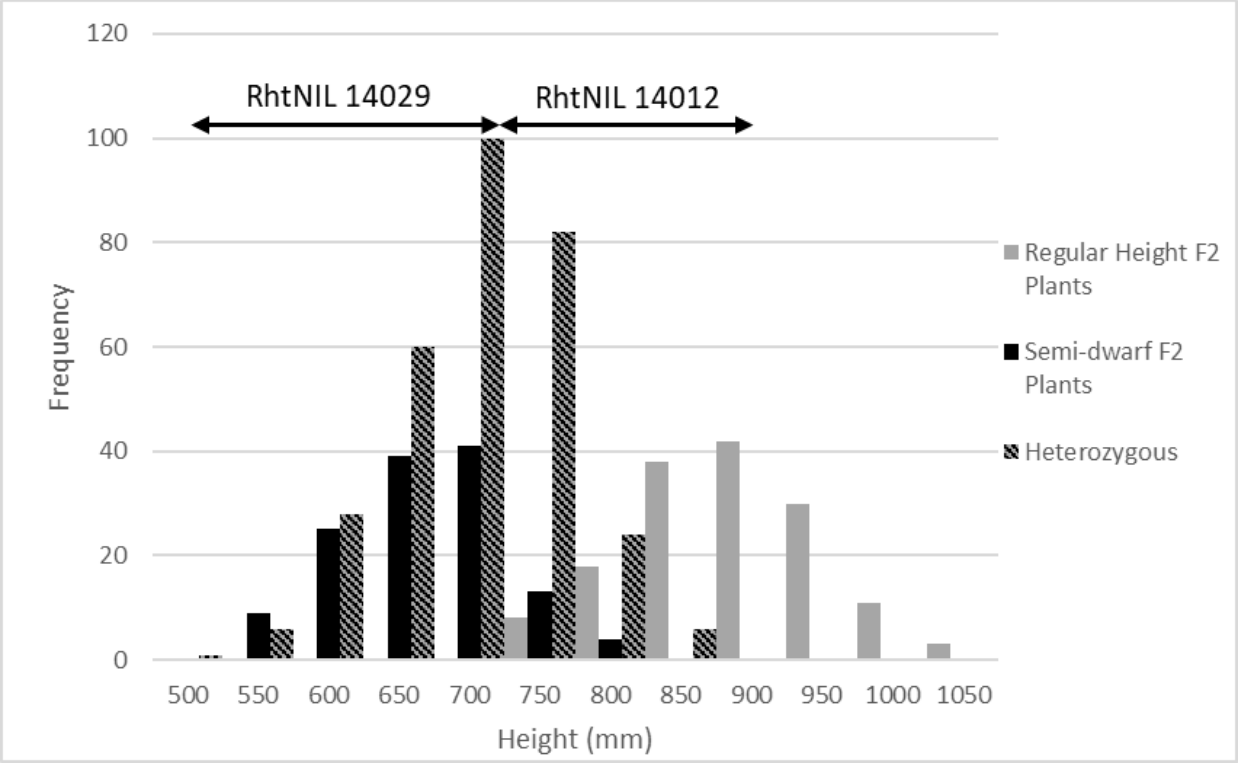

Figure 4.2. Height distribution of 588 F_2 lines, categorized into either homozygous semi-dwarf carrying Rht18 (black), heterozygous (striped), or homozygous regular height not carrying Rht18 (grey) based on the calls of the KASP markers. The range of heights of the parents of the F_2 plants are shown by the black arrows. The heights were measured from the base of the stem to the end of the spike at maturity after being grown in a controlled environment chamber.

clustered along the x-axis, with a high FAM fluorescence value and low HEX florescence value. F_2 plants called as heterozygotes were grouped in a third cluster in the center of the chart with approximately equal HEX and FAM fluorescence values.

In the current study, there was no recombination observed between any of the markers and phenotypic data within the F_2 population. All of the markers tested called each of the F_2 plant samples consistently and the SNP calls for each plant also matched with the phenotypic height data that was measured at maturity. There were 131 SNPs in the 90k chip array that differed between the 12 tall NILs and the 12 short NILs. When placed on a previously published consensus map by Wang et al. (2014b), 126 of the 131 SNPs were located on chromosome 6A, one was found on both chromosome 2A and 2B, one was located on chromosome 2A, and one was located on chromosome 2B (Supplementary Table S1). The other two SNPs were not found on the map by Wang et al. (2014b) but were located on chromosome 6A on a different consensus map (Wen et al. 2017). Of the 126 SNPs located on chromosome 6A by Wang et al. (2014b), 100

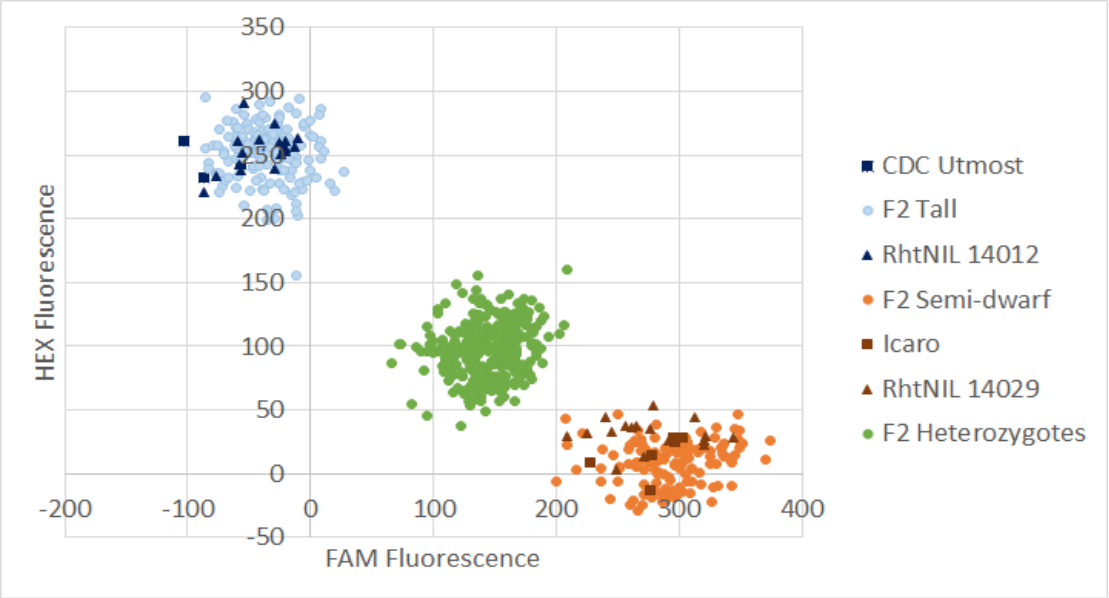

Figure 4.3. IWB52666 KASP marker standardized data categorized into tall (blue), heterozygote (green), or semi-dwarf (orange) clusters. Each individual data point is a fluorescence value (ratio of HEX on the y-axis and FAM on the x-axis) for DNA from F_2 (RhtNIL 14012/RhtNIL14029), RhtNIL 14012, RhtNIL 14029, CDC Utmost, or Icaro plants. The clustering was similar for each of the markers tested.

SNPs were located at the same location at 79.075 cM, and 123 SNPs spanned the 78.502 to 79.394 cM region on chromosome 6A (Supplementary Table S1). Based on the map by Wang et al. (2014b), the SNPs that differed between the tall NILs and short NILs were found near the centromere. Physical locations of the SNPs were determined using the bread wheat iwgsc_refseqv1.0 assembly and the durum wheat (cv. Svevo) RefSeq Release 1.0 assembly. The SNPs that spanned the 78.502 to 79.394 cM region on chromosome 6A cM on the Wang et al. (2014b) consensus map covered 263.4 Mb of the 618.1 Mb chromosome and 364.6 Mb of the 615.7 Mb chromosome on the bread wheat and durum wheat assemblies, respectively.

These same markers were run on 125 previously released cultivars or accessions, consisting of 95 hexaploid wheat, 24 durum, one triticale (x *Triticosecale* Wittmack), one emmer (*Triticum turgidum* spp. *dicoccum*), one khorasan wheat (*Triticum turanicum* Jakubz.), two spelt (*Triticum spelta* L.), and one barley (*Hordeum vulgare* L.) cultivar. The three SNP-based markers IWB52666, IWB39455, and IWA1813 showed the most promising results (Appendix G). Of the 125 accessions, they miscalled 27 as positive for the *Rht18* allele. Of the 27 wheat lines, 23 of them were durum accessions, one was a triticale, one was an emmer, one was a

Khorasan, and one was the hexaploid wheat cultivar Tordo, which was one of the original cultivars carrying the *Rht-B1c* semi-dwarfing allele. The other 98 accessions that were accurately called as not carrying the *Rht18* allele consisted of 94 hexaploid wheat lines, one barley, two spelt, and one durum accession. These three SNPs were found to be within 10.87 Mb of each other based on the iwgsc_refseqv1.0 assembly and within 10.80 Mb of each other based on the RefSeq Release 1.0 assembly. The other SNP markers evaluated had a higher frequency of inaccurate calls in the hexaploid wheat accessions, indicating that they are not as useful in marker assisted selection for the *Rht18* allele.

4.3.4 Phenotypic selection of Rht18 carriers using the distance between the flag leaf and basal spikelet node

There was a highly significant correlation between height and distance from flag leaf to the bottom of the spike with a Pearson's correlation coefficient of $r = 0.7136$ and a $p < 0.0001$. The regression model was found to be strongly significant (Table 4.2). This showed the potential for measuring the approximate height of the wheat plant based on the distance from the ligule of the flag leaf to the bottom of spike (Equation 4.1). Even if the calculated height was not desired, there was a general correlation between the measurements with shorter plants having less distance between the flag leaf ligule and spike than the taller plants.

Table 4.2. The ANOVA table for the regression model to predict the height of a wheat plant based of the distance between the flag leaf ligule to the base of the spike. The data used is specific to the F_2 plants (RhtNIL 14012/RhtNIL 14029), RhtNIL 14012, RhtNIL 14012, and CDC Utmost used in this experiment.

Source	df	Sum of Squares	Mean Square	F value	P value
Model	1	3686172	3686172	652.60	< 0.0001
Error	629	3552855	5648.42		
Corrected Total	630	7239027			

df = degrees of freedom

Equation 4.1. Linear regression equation to calculate the plant height of *Rht18* F_2 plants. The distance measured for the calculated is the distance from the ligule of the flag leaf (FL) to the bottom of the spike.

Plant height (mm) = 671.37 + 1.64*distance from FL ligule to spike base (mm)

4.4 Discussion

4.4.1 F_2 mapping population

In an F_2 population segregating for the *Rht18* allele there was an expected 1:2:1 ratio. This means that that 25% of the F_2 plants would not carry the *Rht18* allele, 50% would be heterozygous, and 25% would carry the *Rht18* allele. In the present study the 1:2:1 ratio was confirmed with the KASP markers calling the 588 F_2 plants 150 homozygous –*Rht18*:307 heterozygous:131 homozygous +*Rht18*.

Rht18 was initially classified as a semi-dominant gene, which means that plants that are heterogynous would have a distinct phenotype and in a range between plants in either homozygous conditions (Haque et al. 2011). Plants heterozygous for *Rht18* have an intermediate height ranging between the semi-dwarf and the regular height parents. *Rht18* has since been categorized as dominant in nature, which means that the heterozygotes plants would have a phenotype similar to the homozygous semi-dwarf plants (Tang 2015; Ford et al. 2018). In the current study, the heterozygote F_2 phenotypes appeared to skew towards the semi-dwarf height, but still had a distinct bell curve shape that was intermediate in height compared to the semi-dwarf and tall F_2 plants.

4.4.2 Validation of Markers

Height is a trait that cannot be phenotypically screened for until late in a plant's life cycle. Therefore, it is important to use marker-assisted selection to help accelerate selection for semi-dwarf wheat genotypes. In the RhtNIL 14029/RhtNIL 14012 F_2 population there were no recombinants between the *Rht18* semi-dwarf allele and any of the KASP markers tested. The selected markers accurately called each of the 24 NILs and 588 F_2 wheat plants. *Rht18* has been previously mapped in durum wheat near the centromere (Ford et al. 2018) on the short arm of chromosome 6A (Haque et al. 2011; Tang 2015; Vikhe et al. 2017). The majority of the 131 markers that differed between the tall NILs and short NILs were found near the centromere of chromosome 6A in the 78.502 to 79.394 cM region. These SNPs spanned approximately 263.4 Mb of the chromosome. In wheat, as well as other plant species, there is a reduced rate of recombination near the centromere, and therefore a relatively low cM Mb^{-1} compared to other areas of the chromosome (Copenhaver et al. 1998; Akhunov et al. 2003). This was found in the current study, where there was approximately 0.89 cM 263.4 Mb^{-1}. Along with reduced recombination, there is also a reduction in gene density and DNA polymorphisms near the

centromere, with the distal areas of a chromosome typically having higher of both (Dvorák et al. 1998; Rustenholz et al. 2011). On the published consensus map by Wang et al. (2014b) there were 2133 SNPs on chromosome 6A. Of these 2133 SNPs, 303 of them located on the 0.89 cM (263.4 Mb) region of the chromosome. The three KASP markers that showed the most promising results after validation were found on the short arm of chromosome 6A when placed on the SNP consensus map by Wang et al. (2014b). They were also found to be physically located within a 10.80 to 10.87 Mb area of the chromosome, which is relatively closer to each other than the other SNPs.

The genotypes of the plants for each of the KASP markers tested matched with the phenotypic height data, with the F_2 plants that were homozygous *Rht18*-positive being distinguishably semi-dwarf and homozygous *Rht18*-negative being distinguishably taller. The heterozygous plants matched the genotype as well, with the height distributed in a bell curve pattern ranging from a semi-dwarf height of 500 mm to a regular height of 840 mm. The *Rht18* gene was previously classified as semi-dominant (Konzak 1988), so it was expected that the heterozygote F_2 plant heights would have fallen in a range somewhere in between the homozygous semi-dwarf and homozygous regular height F_2 plants. Therefore, it was not possible to confirm whether the F_2 plants were truly heterozygous based on their phenotype. To confirm that the markers matched the phenotype, and that the plants were truly heterozygous the $F_{2:3}$ population would have to be grown out. Since all of the markers tested called all 588 F_2 plants the same and passed the chi-square test the $F_{2:3}$ population was not grown out.

Even if a molecular marker works well in a specific population, it is important to test whether it works in a variety of genotypes and is robust enough to be used in different breeding programs. The three SNP-based markers IWB52666, IWB39455, and IWA1813 accurately called 92 of the 93 hexaploid bread wheat accessions that were not known to carry *Rht18* and were the most promising markers. The markers called 22 of the 23 durum accessions as carrying *Rht18* even though they were not known to. These results show that there was potential for the markers IWB52666, IWB39455, and IWA1813 to be used in hexaploid wheat breeding programs. Another study by Vikhe et al. (2017) found that the two SNP markers IAW4371 and TdGA2ox-A9 showed one and no recombinants, in a RIL durum population, respectively, but were present at a high frequency in durum cultivars. The SSR marker S470865SSR4 was not present in either hexaploid or durum wheat and could potentially be used in breeding programs

where *Rht18* screening if desired (Vikhe et al. 2017). These markers were tested on wheat lines and cultivars from different parts of the world as well, and would have to be tested to see if they would be appropriate for use in a Canadian wheat breeding program.

4.4.3 Phenotypic Selection of Rht18 using Flag Leaf to Spike Distance

While using molecular markers to screen for phenotypic traits such as height is important and result in an accelerated rate of accurate selection, phenotypic selection is still a method that is commonly used for most traits. Correlations have been detected between many different crop traits, either for traits that correlate for physical reasons or because the traits are genetically correlated, by either linkage or pleiotropy (Chen and Lübberstedt 2010). There was a strong correlation between peduncle length and the height of F_2 plants carrying *Rht18*. The peduncle length, or the distance from flag leaf to spike was strongly correlated to the plant height of F_2 plants carrying *Rht18*. When grown in a controlled environment some of the *Rht18* carriers had no or a negative distance, where the spike never actually emerged fully from the boot. This made it possible to phenotypically select the semi-dwarf plants without necessarily screening with a molecular marker.

4.5 Conclusions

The chi-square test for plant height of the F_2 mapping population created from the cross RhtNIL 14029/RhtNIL 14012 confirmed it to be an appropriate population to be used for validation of markers for the *Rht18* allele. Based on the 90k SNP data for the 12 *Rht18*-carrying NILs, 12 *Rht18*-non carrying NILs, CDC Utmost, and Icaro it was confirmed that *Rht18* was located on chromosome 6A, in a similar location to earlier reports. The three SNP-based markers IWB52666, IWB39455, and IWA1813 showed promising results for use in screening for the *Rht18* allele in bread wheat, but not in durum wheat. The three markers showed no recombination between each other or the *Rht18* phenotype in both the F_2 mapping population and 92 of the 93 hexaploid bread wheat accessions.

There was a correlation between the distance between the flag leaf ligule and the base of the spike and height in wheat. This allowed for the potential phenotypic screening of lines used in breeding programs.

5.0 General Overview

5.1 General Discussion

Semi-dwarfing genes can benefit a variety of different crops. When bred into plants, these genes result in a shorter stature compared to regular height plants that do not contain the genes. Shortening of plants can reduce the likelihood of stem lodging under certain environmental conditions. Plants that carry semi-dwarfing genes have the potential to increase the HI due to similar or increased grain yield and less total biomass compared to regular height counterparts. In the process of breeding wheat varieties to carry these genes, it is important to evaluate the agronomic and end-use effects of the gene on the plant to check for any potential linkage drag. Locating the gene and validating a molecular marker for the gene will allow for more efficient crossing and increased rate of introgression.

The current research was successful at locating and validating a molecular marker for the dwarfing gene *Rht18* in hexaploid wheat. Linkage drag was also measured in hexaploid wheat lines carrying *Rht18* and there were minimal agronomic and quality effects when compared to NILs of the same cross that do not carry the gene. The location of the *Rht18* gene was determined to be near the centromere of chromosome 6A in wheat, which matches results by previous studies (Haque et al. 2011; Tang 2015; Vikhe et al. 2017; Ford et al. 2018; Grant et al. 2018).

Linkage drag is the negative effects or reduced fitness that is caused by the introduction of genes other than the gene of interest during introgression and backcrossing of the gene. Since *Rht18* was introduced from another wheat species through backcrossing it was important to ensure there was no linkage drag that would cause negative effects on the agronomic or end-use quality factors of the wheat. It has been shown that there is reduced recombination of genes near the centromere of the plant (Copenhaver et al. 1998). Since *Rht18* was identified near the centromere, there is potential that other genes were linked during crossing that was conducted to create the NILs that carry *Rht18*. This means that there may be a higher likelihood of linkage drag associated with this gene. When the NILs carrying *Rht18* were compared to NILs that did not, there were some differences in agronomic and quality traits between the two groupings.

Varying traits between the two NIL groups could have potentially been due to *Rht18* directly by pleiotropy, the linkage of other genes with the *Rht18* gene, or the physical reduction in height.

The *Rht18* gene is a mutation in the coding region of *GA2oxA9* which results in the increased expression of the gene, and subsequently a decrease in bioactive GA production in wheat (Ford et al. 2018). Since reduced bioactive GA in plants reduces stem elongation, the reduced height is a direct result of this gene. Similarly, reduced stem lodging may be due to reduced height. The reduced length of the coleoptile in the *Rht18*-carrying lines was potentially due to the reduced amount of GA and therefore reduced cell elongation in the coleoptile of the plant. Therefore, some of the statistical differences between NILs carrying *Rht18* and not carrying *Rht18* would have resulted from the physiological changes and the reduction in height caused by *Rht18* and not due to linkage drag. One potential effect of linkage drag may have been the linkage of FHB resistance QTLs that were found on chromosome 6A with *Rht18* in wheat. The short NILs had a statistically significant increase in FHB compared to the tall NILs. There are QTLs that effect FHB resistance located on chromosome 6A in a similar location to *Rht18* (Schmolke et al. 2005; Prat 2016). In the current study, the presence of these QTLs was not analyzed, and therefore it was not known whether there was linkage of *Rht18* with genes that may affect FHB presence in the resulting NILs. Increases in FHB also negatively correlated with height, and in one study by Yan et al. 2011 the increase in FHB incidence in semi-dwarf wheat was eliminated when the plants were superficially raised to the same height of the regular height control wheat plants.

There were statistical differences for agronomic traits that were measured in the different NIL groups. Some of these included differences in days to heading, days to maturity, lodging score, FHB infection, test weight, thousand kernel weight and grain yield. There were also differences for most of these traits between individual NILs between each grouping, with some of the individual tall and shorter NILs having had similar measurements. More research would have to be conducted to measure whether the difference in agronomic, quality, and end-use traits between the NILs carrying and not carrying *Rht18* were due to *Rht18* pleiotropic effects, reduced height, or linkage drag. Variations between the short NILs also allowed for selection of semi-dwarf wheat lines that have more desirable agronomic and quality profiles. By having molecular markers that were useable for detection of *Rht18* in hexaploid wheat, there could be an increase in rate and efficiency for screening of wheat lines that carry the gene. These markers could be

useful in a breeding program to introduce the gene that reduced stature of the wheat plant with minimal effects to other traits.

5.2 General Conclusion

The semi-dwarfing gene *Rht18* was classified as GA sensitive and the testing of the short NILs in this study confirmed this. Due to the finding of other studies, GA sensitive wheat cultivars are generally considered to have longer coleoptiles compared to GA insensitive semi-dwarf cultivars, which is significant because it results in better seedling emergence in drier areas where seed must be sown deeper. The short NILs examined in this study had shorter coleoptile lengths than the tall NILs not carrying the *Rht18* dwarfing gene.

Agronomic analysis of short NILs carrying the *Rht18* semi-dwarfing gene from the donor durum cultivar Icaro backcrossed into the recurrent parent CDC Utmost showed that there was reduced height in the resulting lines with minimal linkage of undesirable traits. There was no reduction in seedling emergence or spikes per unit area compared to the parent CDC Utmost or the tall NILs, but there was a slight increase in days to heading and days to maturity. There was a significant reduction in height due to a reduction in spike length and length of all internodes, and a significant reduction in lodging compared to the tall NILs. There was a statistically significant reduction in yield, but an increase in HI compared to the tall NILs. There was also a small, but statistically significant reduction in test weight, thousand kernel weight, spikelets spike^{-1}, seeds spike^{-1}, and seed yield spike^{-1}. Seed quality tests detected higher percent protein and FN, as well as a reduced SDS Sedimentation volume relative to the tall NILs.

KASP molecular markers were successfully developed to differentiate spring wheat lines carrying the *Rht18* semi-dwarfing gene in both heterozygous and homozygous states. The three SNP-based markers IWB52666, IWB39455, and IWA1813 showed promising results for the use of screening for the *Rht18* allele in bread wheat, but not in durum wheat. Validation of the markers in an F_2 population, developed by crossing one of the semi-dwarf NILs and one of the regular height NILs, accurately called all 588 F_2 plants. The markers showed no recombination between each other or the *Rht18* phenotype in both the F_2 mapping population and 92 of the 93 hexaploid bread wheat accessions. Based on the 90k SNP data for the 12 short NILs, 12 tall NILs, CDC Utmost, and Icaro, it was confirmed that *Rht18* was found on the short arm of chromosome 6A.

5.3 Future Work

Wheat carrying other dwarfing genes may have a reduction in coleoptile length and emergence when sown at deeper depth which is required in dry soil conditions. In the current study there was no difference in emergence found between the short and tall NILs tested. Further studies would have to be conducted to determine whether the short NILs would have a reduced emergence if grown in environments that require deeper sowing depths. This could be tested by seeding at various depths in the field or in controlled environment tests. Similarly, further work would have to be conducted to test whether there is a reduced rate of coleoptile growth resulting in a slower rate of emergence, which has been reported in other dwarfed wheats.

Future work is required to determine whether there is a reduction in stem lodging in the short NILs for reasons other than height reduction. The stem diameter, wall thickness, lignin content, and leaf area would have to be measured to determine whether wheat carrying *Rht18* has resistance to stem lodging for reasons other than reduction in height. To determine any difference in root lodging between the short and tall NILs further studies could be conducted to test whether there is any difference in root characteristics that may result in reduced root anchorage, and therefore potentially an increase in root lodging.

It is uncertain whether the increase in susceptibility of the short NILs to FHB is due to phenotypical characteristics such as a reduced height, or due to genetic conditions. In the current study no markers for FHB QTLs were tested on the short NILs, and this would be important work to carry out in the future to understand the cause of increased FHB susceptibility. It was also not determined whether this increase in susceptibility is due to a decrease in Type 1 or Type 2 resistance. To understand the cause of susceptibility of *Rht18* carrying wheat to FHB, and potentially increase the resistance to the disease, tests would need to be conducted.

There were statistical differences in protein, FN, and SDS sedimentation volumes between the short and tall NILs, but the mean differences were small. To determine whether the difference in quality would translate into differences in end-use quality characteristics further baking tests would have to be conducted.

When the 125 cereal accessions were screened using the potential SNP markers for Rht18, all the hexaploid wheat accessions read negative for the markers except for Tordo. Tordo is known to carry the *Rht-B1c* gene, but further tests would be needed to analyze why Tordo read positive for *Rht18*, and if there are any functional correlation between the two dwarfing genes.

The potential SNPs read negative for *Rht18* in all of the hexaploid wheat accessions except for one, but positive for the gene in durum. Further studies would have to be conducted to determine why.

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7.0 Appendices

Appendix A. Average coleoptile length (mm), primary root length (mm), and total root length (mm). Measurements were taken for the recurrent parent CDC Utmost, the three checks Carberry, Glenn, and CDC Go, the individual tall (*Rht18* not present, "-" behind the name) NILs of Utmost*6/Icaro, and the individual short (*Rht18* present, "+" behind the name) NILs of Utmost*6/Icaro.

Line	n	Coleoptile length (mm)	Primary root length (mm)	Total root length (mm)
CDC Utmost	3	67.3	121.2	287.3
Carberry	3	56.8	136.3	361.7
Glenn	3	68.5	149.3	338.7
CDC Go	3	57.5	145.6	389.7
RhtNIL 14003 (-)	3	64.3	113.4	272.7
RhtNIL 14004 (-)	3	68.4	123.6	316.1
RhtNIL 14005 (+)	3	57.2	115.0	285.0
RhtNIL 14006 (-)	3	64.2	115.4	285.7
RhtNIL 14007 (-)	3	59.9	122.0	289.4
RhtNIL 14010 (-)	3	62.8	124.0	289.2
RhtNIL 14011 (-)	3	63.2	123.9	300.1
RhtNIL 14012 (-)	3	62.9	113.2	266.4
RhtNIL 14013 (-)	3	62.4	119.7	256.1
RhtNIL 14014 (-)	3	64.5	117.4	271.8
RhtNIL 14015 (+)	3	65.9	125.2	309.6
RhtNIL 14016 (+)	3	57.4	128.6	308.7
RhtNIL 14017 (-)	3	64.0	121.5	296.5
RhtNIL 14018 (-)	3	65.4	109.9	266.9
RhtNIL 14019 (+)	3	57.4	125.7	315.0
RhtNIL 14020 (+)	3	60.4	129.6	323.0
RhtNIL 14021 (+)	3	64.3	106.0	268.7
RhtNIL 14022 (+)	3	52.9	107.2	228.8
RhtNIL 14023 (+)	3	57.5	125.4	286.8
RhtNIL 14024 (+)	3	55.5	112.3	254.6
RhtNIL 14027 (+)	3	55.3	116.7	252.4
RhtNIL 14028 (+)	3	59.3	119.4	293.5
RhtNIL 14029 (+)	3	60.4	123.4	275.6
RhtNIL 14032 (-)	3	67.7	115.5	268.6
SEM		**2.41**	**7.61**	**27.10**
CV		**8.95**	**12.07**	**17.54**

Significance	****	**	*

Note: Data are the mean estimates of each cultivar or line. SEM are the standard error means. *, **, ***, ****: significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$. ns: not statistically significant.

Appendix B. Averages for field agronomic measurements (days to heading, days to maturity, emergence count, spike count, and lodging score) for KCRF, Brown, and Goodale locations combined in 2016 and 2017. Measurements were taken for the recurrent parent CDC Utmost, the three checks Carberry, Glenn, and CDC Go, the individual tall (*Rht18* not present, "-" behind the name) NILs of Utmost*6/Icaro, and the individual short (*Rht18* present, "+" behind the name) NILs of Utmost*6/Icaro.

Line	n	Heading (days)	Maturity (days)	Emergence (plants m^{-2})	Spike Count (spike m^{-2})	Lodging (Belgian scale)
CDC Utmost	24	53.2	92.2	239.2	579.2	1.67
Carberry	24	49.9	95.1	239.2	572.8	0.22
Glenn	24	50.4	94.8	244.1	585.5	0.59
CDC Go	24	49.0	90.8	235.5	589.8	0.27
RhtNIL 14003 (-)	24	52.9	91.0	249.1	532.9	0.68
RhtNIL 14004 (-)	24	52.8	91.7	238.9	570.5	0.58
RhtNIL 14005 (+)	24	55.9	93.7	238.4	580.5	0.20
RhtNIL 14006 (-)	24	53.3	91.5	244.0	550.5	0.72
RhtNIL 14007 (-)	24	53.1	91.0	233.2	536.0	0.80
RhtNIL 14010 (-)	24	53.3	91.1	241.8	587.1	1.09
RhtNIL 14011 (-)	24	52.5	89.9	245.1	554.8	1.22
RhtNIL 14012 (-)	24	53.2	91.3	246.0	543.7	0.99
RhtNIL 14013 (-)	24	53.1	91.0	255.0	541.3	1.07
RhtNIL 14014 (-)	24	53.6	91.5	253.4	571.4	1.67
RhtNIL 14015 (+)	24	56.0	93.5	233.0	551.9	0.20
RhtNIL 14016 (+)	24	56.1	94.3	236.3	566.7	0.20
RhtNIL 14017 (-)	24	53.8	91.7	246.3	554.0	0.83
RhtNIL 14018 (-)	24	53.0	91.3	249.2	548.1	0.71
RhtNIL 14019 (+)	24	55.5	94.2	243.2	554.0	0.20
RhtNIL 14020 (+)	24	55.4	93.7	242.0	542.7	0.20
RhtNIL 14021 (+)	24	55.1	93.0	245.0	578.8	0.20
RhtNIL 14022 (+)	24	55.7	93.3	248.3	588.3	0.20
RhtNIL 14023 (+)	24	55.3	93.0	244.9	586.3	0.20
RhtNIL 14024 (+)	24	55.0	92.5	263.8	572.6	0.20
RhtNIL 14027 (+)	24	54.5	93.3	243.8	596.7	0.20
RhtNIL 14028 (+)	24	54.8	92.9	238.7	563.7	0.20

RhtNIL 14029 (+)	24	56.1	94.5	238.3	546.4	0.20
RhtNIL 14032 (-)	24	53.0	91.0	245.1	547.71	1.17
SEM		**1.23**	**1.07**	**10.32**	**21.17**	**0.267**
CV		**5.91**	**3.44**	**17.00**	**13.33**	**167.83**
Significance		********	********	ns	******	********

Note: Data are the mean estimates of each cultivar or line. SEM are the standard error means. *, **, ***, ****: significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$. Ns: not statistically significant. Lodging was only present at Goodale and KCRF in 2016 and Goodale in 2017, and varied in severity between blocks.

Appendix C. Average Fusarium head blight (FHB) percentage on wheat spikes based on the visual rating index (VRI) for Carman, Manitoba combined in 2015, 2016, and 2017. Measurements were taken for the recurrent parent CDC Utmost, the three checks Carberry, Glenn, and CDC Go, the individual tall (*Rht18* not present, "-" behind the name) NILs of Utmost*6/Icaro, and the individual short (*Rht18* present, "+" behind the name) NILs of Utmost*6/Icaro.

Line	n	FHB (%)
CDC Utmost	4	46.7
Carberry	4	15.2
Glenn	4	13.2
CDC Go	4	67.9
RhtNIL 14003 (-)	4	49.5
RhtNIL 14004 (-)	4	50.0
RhtNIL 14005 (+)	4	66.2
RhtNIL 14006 (-)	4	42.0
RhtNIL 14007 (-)	4	46.7
RhtNIL 14010 (-)	4	23.7
RhtNIL 14011 (-)	4	41.2
RhtNIL 14012 (-)	4	35.0
RhtNIL 14013 (-)	4	50.7
RhtNIL 14014 (-)	4	32.7
RhtNIL 14015 (+)	4	79.5
RhtNIL 14016 (+)	4	69.2
RhtNIL 14017 (-)	4	43.7
RhtNIL 14018 (-)	4	47.5
RhtNIL 14019 (+)	4	55.2
RhtNIL 14020 (+)	4	69.2
RhtNIL 14021 (+)	4	68.2
RhtNIL 14022 (+)	4	70.7
RhtNIL 14023 (+)	4	60.5
RhtNIL 14024 (+)	4	75.2
RhtNIL 14027 (+)	4	67.0
RhtNIL 14028 (+)	4	64.5
RhtNIL 14029 (+)	4	61.2
RhtNIL 14032 (-)	4	51.7
AC Morse	4	55.0
AC Vista	4	52.5
CDC Teal	4	61.7
AC Cora	4	17.5
FHB 37	4	13.7

5602 HR	4	20.2
AAC Tenacious	2	6.0
SEM		**12.42**
CV		**314.78**
Significance		********

Note: Data are the mean estimates of each cultivar or line. SEM are the standard error means. *, **, ***, ****: significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$. Ns: not statistically significant.

Appendix D. Average measurements of plant height, spike length, top internode length (I1L), penultimate or second internode length (I2L), third internode length (I3L), fourth internode length (I4L), and bottom or fifth internode length (I5L) for KCRF, Brown, and Goodale locations combined in 2016 and 2017. Measurements were taken for the recurrent parent CDC Utmost, the three checks Carberry, Glenn, and CDC Go, the individual tall (*Rht18* not present, "-" behind the name) NILs of Utmost*6/Icaro, and the individual short (*Rht18* present, "+" behind the name) NILs of Utmost*6/Icaro.

Line	n	Plant Height (cm)	Spike Length (mm)	I1L (mm)	I2L (mm)	I3L (mm)	I4L (mm)	I5L (mm)
CDC Utmost	24	92.8	78.0	373.6	232.0	155.8	101.0	29.6
Carberry	24	83.2	75.1	364.3	195.5	126.0	86.1	28.5
Glenn	24	92.3	79.1	387.1	218.4	147.0	97.1	27.7
CDC Go	24	83.1	74.5	358.0	193.4	121.5	81.4	29.7
RhtNIL 14003 (-)	24	91.1	77.6	366.3	226.2	156.6	101.5	29.9
RhtNIL 14004 (-)	24	92.1	76.7	363.7	229.9	156.3	102.4	30.6
RhtNIL 14005 (+)	24	76.8	77.3	281.3	186.0	135.7	88.0	28.2
RhtNIL 14006 (-)	24	90.6	78.3	363.7	225.0	151.0	97.0	26.8
RhtNIL 14007 (-)	24	90.5	77.7	360.9	222.2	149.8	99.1	26.8
RhtNIL 14010 (-)	24	93.1	77.4	373.7	233.9	157.0	97.9	30.0
RhtNIL 14011 (-)	24	91.1	77.3	366.8	225.7	148.8	98.0	28.7
RhtNIL 14012 (-)	24	93.2	77.1	369.7	233.6	158.4	103.2	29.8
RhtNIL 14013 (-)	24	91.3	77.4	358.5	226.7	153.4	99.4	29.1
RhtNIL 14014 (-)	24	91.7	77.8	368.2	230.6	154.2	98.2	28.4
RhtNIL 14015 (+)	24	77.1	78.3	282.9	186.7	135.4	87.9	28.0
RhtNIL 14016 (+)	24	77.0	77.2	278.6	184.6	132.7	88.6	28.5
RhtNIL 14017 (-)	24	94.4	77.7	369.0	237.1	161.8	106.3	31.8
RhtNIL 14018 (-)	24	91.1	77.0	363.2	222.6	150.0	100.2	28.8
RhtNIL 14019 (+)	24	76.1	76.9	273.4	185.0	133.5	85.7	28.7
RhtNIL 14020 (+)	24	75.8	77.1	281.4	185.7	131.1	85.3	26.0
RhtNIL 14021 (+)	24	75.8	77.0	282.7	183.1	133.1	84.8	28.1
RhtNIL 14022 (+)	24	74.9	76.3	282.0	184.6	133.7	85.8	27.2
RhtNIL 14023 (+)	24	74.2	75.1	266.5	180.1	130.1	84.0	27.8
RhtNIL 14024 (+)	24	74.5	75.5	272.1	179.8	129.5	82.8	26.6
RhtNIL 14027 (+)	24	74.4	76.1	268.2	179.5	128.3	82.6	27.3

RhtNIL 14028 (+)	24	73.8	75.7	269.6	178.2	129.6	82.0	26.8
RhtNIL 14029 (+)	24	76.0	77.0	277.0	184.2	135.1	89.4	29.6
RhtNIL 14032 (-)	24	90.4	76.3	358.0	221.1	151.4	99.2	31.2
SEM		**3.48**	**4.17**	**8.48**	**8.32**	**12.67**	**13.53**	**2.37**
CV		**5.91**	**6.66**	**15.73**	**15.14**	**16.35**	**20.66**	**23.09**
Significance		********	********	********	********	********	********	*****

Note: Data are the mean estimates of each cultivar or line. SEM are the standard error means. *, **, ***, ****: significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$. Ns: not statistically significant.

Appendix E. Average post-harvest agronomic measurements (yield, test weight (TW), thousand kernel weight (TKW), number of spikelets spike^{-1}, number of seeds spike^{-1}, seed yield spike^{-1}, and harvest index (HI)) for KCRF, Brown, and Goodale locations combined in 2016 and 2017. Measurements were taken for the recurrent parent CDC Utmost, the three checks Carberry, Glenn, and CDC Go, the individual tall (*Rht18* not present, "-" behind the name) NILs of Utmost*6/Icaro, and the individual short (*Rht18* present, "+" behind the name) NILs of Utmost*6/Icaro.

Line	n	Yield (kg ha^{-1})	TW (kg hl^{-1})	TKW (g)	Spikelets Spike^{-1}	Seeds Spike^{-1}	Seed Yield Spike (g) $^{-1}$	HI
CDC Utmost	24	4475	79.4	34.4	15.2	26.3	0.896	0.383
Carberry	24	4542	80.9	33.9	14.0	23.8	0.795	0.397
Glenn	24	4472	83.0	32.9	14.2	24.4	0.788	0.353
CDC Go	24	4632	80.0	37.9	13.3	23.4	0.863	0.412
RhtNIL 14003 (-)	24	4357	79.0	34.1	15.2	26.7	0.885	0.378
RhtNIL 14004 (-)	24	4472	79.0	33.9	15.0	26.5	0.878	0.379
RhtNIL 14005 (+)	24	4435	78.0	33.2	14.8	23.8	0.770	0.382
RhtNIL 14006 (-)	24	4600	79.6	34.7	15.5	25.9	0.875	0.382
RhtNIL 14007 (-)	24	4480	79.4	34.5	15.3	25.5	0.863	0.378
RhtNIL 14010 (-)	24	4545	79.4	34.1	15.0	25.6	0.854	0.375
RhtNIL 14011 (-)	24	4276	79.2	33.9	14.8	25.9	0.852	0.386
RhtNIL 14012 (-)	24	4499	79.4	34.2	15.0	25.8	0.866	0.376
RhtNIL 14013 (-)	24	4297	79.3	34.2	15.0	24.5	0.817	0.353
RhtNIL 14014 (-)	24	4389	78.9	33.7	15.3	27.8	0.883	0.381
RhtNIL 14015 (+)	24	4377	78.3	33.0	15.2	24.3	0.788	0.383
RhtNIL 14016 (+)	24	4308	78.0	33.2	15.0	24.0	0.773	0.381
RhtNIL 14017 (-)	24	4444	79.2	33.6	15.2	25.8	0.849	0.372
RhtNIL 14018 (-)	24	4451	79.6	34.5	15.1	25.5	0.860	0.378
RhtNIL 14019 (+)	24	4297	77.7	33.3	14.9	23.4	0.763	0.379
RhtNIL 14020 (+)	24	4321	77.9	33.4	14.8	23.7	0.770	0.374
RhtNIL 14021 (+)	24	4386	77.8	33.2	14.9	24.6	0.796	0.396
RhtNIL 14022 (+)	24	4378	77.9	33.4	14.9	23.8	0.770	0.387
RhtNIL 14023 (+)	24	4291	78.1	32.7	14.6	23.6	0.743	0.392
RhtNIL 14024 (+)	24	4246	78.1	32.7	14.5	23.6	0.747	0.387
RhtNIL 14027 (+)	24	4252	77.9	32.3	14.6	22.8	0.729	0.385

RhtNIL 14028 (+)	24	4296	77.7	32.8	14.4	23.2	0.747	0.385
RhtNIL 14029 (+)	24	4233	78.1	33.6	15.0	23.9	0.778	0.380
RhtNIL 14032 (-)	24	4270	79.2	34.0	15.0	25.3	0.843	0.378
SEM		**320.4**	**2.83**	**0.84**	**0.30**	**2.32**	**0.0896**	**0.0147**
CV		**13.75**	**3.93**	**5.47**	**5.80**	**16.98**	**16.78**	**11.42**
Significance		********	********	********	********	********	********	********

Note: Data are the mean estimates of each cultivar or line. SEM are the standard error means. *, **, ***, ****: significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$. Ns: not statistically significant.

Appendix F. Average seed quality measurements (protein, Falling Number (FN), and SDS Sedimentation (SED)) for KCRF, Brown, and Goodale locations combined in 2016 and 2017. Measurements were taken for the recurrent parent CDC Utmost, the three checks Carberry, Glenn, and CDC Go, the individual tall (*Rht18* not present, "-" behind the name) NILs of Utmost*6/Icaro, and the individual short (*Rht18* present, "+" behind the name) NILs of Utmost*6/Icaro.

Line	n	Protein (%)	FN (seconds)	SED (ml)
CDC Utmost	24	16.38	437.7	80.3
Carberry	24	16.29	387.8	76.8
Glenn	24	16.43	414.0	80.7
CDC Go	24	16.51	418.5	76.5
RhtNIL 14003 (-)	24	16.05	421.7	79.9
RhtNIL 14004 (-)	24	16.13	421.3	80.6
RhtNIL 14005 (+)	24	16.59	457.6	78.4
RhtNIL 14006 (-)	24	16.17	438.8	80.0
RhtNIL 14007 (-)	24	16.14	437.1	80.0
RhtNIL 14010 (-)	24	16.49	449.5	82.5
RhtNIL 14011 (-)	24	16.58	447.2	82.2
RhtNIL 14012 (-)	24	16.40	443.5	82.3
RhtNIL 14013 (-)	24	16.28	442.3	80.7
RhtNIL 14014 (-)	24	16.51	438.8	78.9
RhtNIL 14015 (+)	24	16.65	464.8	79.6
RhtNIL 14016 (+)	24	16.75	460.5	78.9
RhtNIL 14017 (-)	24	16.65	450.2	83.1
RhtNIL 14018 (-)	24	16.07	441.6	82.6
RhtNIL 14019 (+)	24	16.45	457.5	77.7
RhtNIL 14020 (+)	24	16.43	454.0	77.2
RhtNIL 14021 (+)	24	16.10	451.9	76.2
RhtNIL 14022 (+)	24	16.11	457.5	76.6
RhtNIL 14023 (+)	24	16.44	458.3	78.1
RhtNIL 14024 (+)	24	16.52	460.6	79.8
RhtNIL 14027 (+)	24	16.45	451.1	78.9
RhtNIL 14028 (+)	24	16.53	455.5	78.8
RhtNIL 14029 (+)	24	16.51	446.8	80.0
RhtNIL 14032 (-)	24	16.63	453.8	82.7
SEM		**0.0136**	**6.58**	**1.30**
CV		**2.81**	**6.40**	**5.41**
Significance		********	********	********

Note: Data are the mean estimates of each cultivar or line. SEM are the standard error means. *, **, ***, ****: significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$. Ns: not statistically significant.

Appendix G. Screening various crop kind cultivars for the *Rht18* allele using SNP-based KASP markers.

Accession	Crop Kind	*Rht18*	IWB52666	IWB39455	IWA1813
Icaro	Durum	+	A	A	A
CDC Utmost	Spring Wheat	-	B	B	B
AC Barrie	Spring Wheat	-	B	B	B
AC Abbey	Spring Wheat	-	B	B	B
CDC Bounty	Spring Wheat	-	B	B	B
AC Eatonia	Spring Wheat	-	B	B	B
AC Cadillac	Spring Wheat	-	B	B	B
Lillian	Spring Wheat	-	B	B	B
AC Elsa	Spring Wheat	-	B	B	B
Lovitt	Spring Wheat	-	B	B	B
Harvest	Spring Wheat	-	B	B	B
McKenzie	Spring Wheat	-	B	B	B
CDC Imagine	Spring Wheat	-	B	B	B
Prodigy	Spring Wheat	-	B	B	B
AC Intrepid	Spring Wheat	-	B	B	B
AC Splendor	Spring Wheat	-	B	B	B
Journey	Spring Wheat	-	B	B	B
Superb	Spring Wheat	-	B	B	B
CDC Teal	Spring Wheat	-	B	B	B
CDC Merlin	Spring Wheat	-	B	B	B
5500HR	Spring Wheat	-	B	B	B
CDC Osler	Spring Wheat	-	B	B	B
5600HR	Spring Wheat	-	B	B	B
CDC Go	Spring Wheat	-	B	B	B
5601HR	Spring Wheat	-	B	B	B
CDC Alsask	Spring Wheat	-	B	B	B
Roblin	Spring Wheat	-	B	B	B
PT559	Spring Wheat	-	B	B	B
Katepwa	Spring Wheat	-	B	B	B
AC Crystal	Spring Wheat	-	B	B	B
Marquis	Spring Wheat	-	B	B	B
AC Foremost	Spring Wheat	-	B	B	B
Red Fife	Spring Wheat	-	B	B	B
AC Taber	Spring Wheat	-	B	B	B
5700PR	Spring Wheat	-	B	B	B
Kyle	Durum	-	A	A	A
5701PR	Spring Wheat	-	B	B	B
AC Avonlea	Durum	-	A	A	A
AC Morse	Durum	-	A	A	A
Napoleon	Durum	-	A	A	A

AC Vista	Spring Wheat	-	B	B	B
AC Navigator	Durum	-	A	A	A
Snowbird	Spring Wheat	-	B	B	B
AC Andrew	Spring Wheat	-	B	B	B
Glenlea	Spring Wheat	-	B	B	B
CDC Kendall	Barley	-	B	B	B
CDC Rama	Spring Wheat	-	B	B	B
CDC Zorba	Spelt	-	B	B	B
CDC Walrus	Spring Wheat	-	B	B	B
Infinity	Spring Wheat	-	B	B	B
Waskada	Spring Wheat	-	B	B	B
CDC Abound	Spring Wheat	-	B	B	B
5602HR	Spring Wheat	-	B	B	B
Alvena	Spring Wheat	-	B	B	B
5702PR	Spring Wheat	-	B	B	B
Goodeve	Spring Wheat	-	B	B	B
Snowstar	Spring Wheat	-	B	B	B
Helios	Spring Wheat	-	B	B	B
Bhishaj	Spring Wheat	-	B	B	B
Kane	Spring Wheat	-	B	B	B
Strongfield	Durum	-	A	A	A
Somerset	Spring Wheat	-	B	B	B
Commander	Durum	-	A	A	A
Unity	Spring Wheat	-	B	B	B
CDC Verona	Durum	-	A	A	A
Fieldstar	Spring Wheat	-	B	B	B
Eurostar	Durum	-	A	A	A
5603HR	Spring Wheat	-	B	B	B
Minnedosa	Spring Wheat	-	B	B	B
859CL (WR859CL)	Spring Wheat	-	B	B	B
Shaw	Spring Wheat	-	B	B	B
Stettler	Spring Wheat	-	B	B	B
Glenn	Spring Wheat	-	B	B	B
Burnside	Spring Wheat	-	B	B	B
Carberry	Spring Wheat	-	B	B	B
Glencross	Spring Wheat	-	B	B	B
Muchmore	Spring Wheat	-	B	B	B
CDN Bison	Spring Wheat	-	B	B	B
5604HR CL	Spring Wheat	-	B	B	B
Brigade	Durum	-	A	A	A
CDC Stanley	Spring Wheat	-	B	B	B
CDC Kernen	Spring Wheat	-	B	B	B
CDC Origin	Spelt	-	B	B	B

CDC VR Morris	Spring Wheat	-	B	B	B
CDC Thrive	Spring Wheat	-	B	B	B
CDC Plentiful	Spring Wheat	-	B	B	B
Enterprise	Durum	-	A	A	A
SY985	Spring Wheat	-	B	B	B
CDC NRG003	Spring Wheat	-	B	B	B
Vesper	Spring Wheat	-	B	B	B
NRG010	Spring Wheat	-	B	B	B
CDC Vivid	Durum	-	A	A	A
Conquer	Spring Wheat	-	B	B	B
Transcend	Durum	-	A	A	A
Sadash	Spring Wheat	-	B	B	B
Cardale	Spring Wheat	-	B	B	B
Pasteur	Spring Wheat	-	B	B	B
Bunker	Triticale	-	A	A	A
Whitehawk	Spring Wheat	-	B	B	B
AC Cora	Spring Wheat	-	B	B	B
Enchant	Spring Wheat	-	B	B	B
Crocus	Spring Wheat	-	B	B	B
AAC Redwater	Spring Wheat	-	B	B	B
Dragon	Khorasan (Tetraploid)	-	A	A	A
CDC Titanium	Spring Wheat	-	B	B	B
Tordo	Spring Wheat	-	A	A	A
09PUFF5	Tetraploid	-	A	A	A
Vernal	Emmer	-	A	A	A
12Toing12	Hexaploid	-	B	B	B
WAX99027	Hexaploid	-	B	B	B
Alsen	Spring Wheat	-	B	B	B
LTDORM82	Hexaploid	-	B	B	B
Neepawa	Spring Wheat	-	B	B	B
CDC Credence	Durum	-	A	A	A
RL4137	Spring Wheat	-	B	B	B
CDC Terrain	Spring Wheat	-	B	B	B
CDC Desire	Durum	-	A	A	A
CDC Throttle	Spring Wheat	-	B	B	B
CDC Fortitude	Durum	-	A	A	A
Kofa	Durum	-	A	A	A
CDC Carbide	Durum	-	A	A	A
W9262-260D3	Durum	-	A	A	A
CDC Precision	Durum	-	A	A	A
UC1113	Durum	-	B	B	B
CDC Dynamic	Durum	-	A	A	A
CDC Alloy	Durum	-	A	A	A

Note: A=same allele as Icaro (+*Rht18*), B=same allele as CDC Utmost (-*Rht18*)